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Prognostic value of cerebrospinal fluid tumor cell count in leptomeningeal disease from solid tumors

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Case Report

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Abstract

Purpose: Treatment decisions for leptomeningeal disease (LMD) rely on patient risk stratification, since clinicians lack objective prognostic tools. The introduction of rare cell capture technology for identification of cerebrospinal fluid tumor cells (CSF-TCs) improved the sensitivity of LMD diagnosis, but prognostic value is unknown. This study assesses the prognostic value of CSF-TC density in patients with LMD from solid tumors.

Methods: We conducted a retrospective cohort study of patients with newly diagnosed or previously treated LMD from a single institution who had CNSide assay testing for CSF-TCs from 2020 to 2023. Univariable and multivariable survival analyses were conducted with Cox proportional-hazards modeling. Maximally-selected rank statistics were used to determine an optimal cutpoint for CSF-TC density and survival.

Results: Of 31 patients, 29 had CSF-TCs detected on CNSide. Median (interquartile range [IQR]) CSF-TC density was 67.8 (4.7-639) TCs/mL. CSF cytology was positive in 16 of 29 patients with positive CNSide (CNSide diagnostic sensitivity = 93.5%, negative predictive value = 85.7%). Median (IQR) survival from time of CSF-TC detection was 176 (89-481) days. On univariable and multivariable analysis, CSF-TC density was significantly associated with survival. An optimal cutpoint for dichotomizing survival by CSF-TC density was 19.34 TCs/mL. The time-dependent sensitivity and specificity for survival using this stratification were 76% and 67% at 6 months and 65% and 67% at 1 year, respectively.

Conclusions: CSF-TC density may carry prognostic value in patients with LMD from solid tumors. Integrating CSF-TC density into LMD patient risk-stratification may help guide treatment decisions.

Introduction

Leptomeningeal disease (LMD) is clinically detected in 5 to 10% of patients with solid tumors, while 20 to 30% of patients with metastatic disease and neurologic symptoms harbor LMD at autopsy [1]. While recent therapeutic advances have improved patient outcomes [2], objective tools for prognostication in patients with LMD are lacking. The recent introduction of rare cell capture technology for identification of cerebrospinal fluid tumor cells (CSF-TCs) improved the sensitivity of LMD diagnosis [3], and one assay subsequently demonstrated an association with survival [4]. However, this assay is reliant upon binding epithelial cell adhesion molecule (EpCAM), which is not universally expressed, and the survival association was limited to patients with newly diagnosed LMD [4].

The CNSide™ assay (formerly Biocept) uses a biotin-tagged antibody cocktail against both EpCAM and non-EpCAM epithelial and mesenchymal antigens to more efficiently detect circulating TCs. CNSide has demonstrated improved diagnostic characteristics to gold standard techniques, but its prognostic value has not been tested[5]. We hypothesized that in an unselected population of patients with newly diagnosed or previously treated LMD from solid tumors, CSF-TC density from the CNSide assay holds

prognostic value. The objective of this work was to identify a prognostic tool applicable to a clinically representative set of patients with LMD.

Methods

Patients

This was a single center, IRB approved, retrospective cohort study of all patients who had undergone CNSide testing for known or suspected LMD between May 2020 and June 2023. The study followed STROBE reporting guidelines [6]. Eligible patients were age 18 years or older and had a histologic proven solid tumor with known or suspected active LMD, regardless of prior treatment status. Patients were pathologically positive for LMD based on CSF cytology or CNSide with CSF-TCs, and were clinically positive based on clinical findings and neuroimaging [7]. CNSide CSF samples were obtained via lumbar puncture, VP shunt, or Ommaya reservoir. CSF-TC density was defined as TCs per 1 mL of CSF. For patients with multiple CNSide tests, the first positive test was used.

Demographic and clinical parameters at time of CNSide were collected from the medical record, including CSF cytology and protein, Karnofsky performance status (KPS), radiographic LMD sites, radiographic LMD type [7], hydrocephalus, oncologic therapies, and systemic disease status. Systemic disease status was classified as active (progressive) or stable via combined radiologist and clinician interpretation of non-CNS disease within 1 month of CNSide.

Statistical Analysis

Survival analysis was conducted for patients with CSF-TCs on CNSide. Median, 6-month, and 1-year overall survival was calculated using Kaplan-Meier estimation. Univariable and multivariable survival analyses were conducted using Cox proportional-hazards regression modeling. The primary outcome measure was the univariable hazard ratio (HR) for survival as predicted by CSF-TC density. On an exploratory basis, other independent variables were tested without multiple comparison correction, and a multivariable model was constructed using CSF-TC density and variables previously identified as prognostic in LMD: KPS, CSF protein, systemic disease status, and age [8–11]. Assumptions of proportional hazards and non-linearity were tested. KPS was dichotomized (KPS $\geq$80 vs < 80) due to proportional hazard violation and a low incidence of KPS $\leq$60. Non-linear variables (CSF-TC density and CSF protein) were log transformed.

For clinical interpretability, we partitioned patients into low- and high-risk CSF-TC density groups using maximally-selected rank statistics, thus determining an optimal cutpoint for CSF-TCs and survival [12]. The resultant *P*-value is not a true type I error rate and is not reported [13]. Time-dependent receiver operating characteristic curve analysis was performed to report the sensitivity and specificity for the CSF-TC density cutpoint relating to 6-month and 1-year survival [14]. Statistical analyses were performed in R, version 4.2.2 (R Project for Statistical Computing). A threshold of 0.05 was used for statistical

significance. Statistics are reported with 95% confidence intervals (CIs), except for median statistics reported with interquartile range (IQR). Missing data was excluded from all analyses.

Results

Patients

Of 43 patients with CNSide, 31 were clinically or radiographically positive for LMD, including 29 with CSF-TCs detected by CNSide (CNSide diagnostic sensitivity = 93.5%, negative predictive value = 85.7%, specificity = 100%). Of the 29 patients with CSF-TCs, the primary cancer types were breast (14 patients) and non-small cell lung cancer (12 patients) (Table 1).

Table 1

Primary Demographic and Clinical Characteristics of 29 Patients with CSF-TCs. Cytology, protein, glucose, and CNSide were performed on CSF acquired from the same procedure. Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range; KPS, Karnofsky performance status; LMD, leptomeningeal disease; TC, tumor cells.

Characteristic	No. (%) of patients
Sex	
Female	23 (79.3)
Male	6 (20.7)
Age, median (IQR) [range], y	57.3 (45.8–64.2) [34.7–77.7]
Primary cancer type, histologic finding	
Breast	14 (48.3)
Lung, non-small cell	12 (41.4)
Other	3 (10.3)
LMD radiographic pattern	
A (linear)	14 (48.3)
B (nodular)	7 (24.1)
C (both)	5 (17.2)
D (none)	3 (10.3)
LMD radiographic sites	
Brain	9 (31.0)
Spine	4 (13.8)
Both	13 (44.8)
None	3 (10.3)
Time from LMD diagnosis to CNSide, median (IQR) [range], d	21 (6-123) [0-2012]
KPS at CNSide	
≥80	18 (64.3)
<80	10 (35.7)
Cytology	
Positive	16 (55.2)
Negative	13 (44.8)

Characteristic	No. (%) of patients
CSF protein, median (IQR) [range], mg/dL	58.5 (44.3-145.3) [33–348]
CSF glucose, median (IQR) [range], mg/dL	55.0 (33.5–61.0) [13.0–80.0]
CNSide CSF-TC density, median (IQR) [range], TCs/mL	67.8 (4.7–639) [0.2-9438.8]
CNSide CSF volume, median (IQR) [range], mL	6.8 (6.6–7.4) [0.8-8.0]
Hydrocephalus at time of CNSide	
Yes	26 (89.7)
No	3 (10.3)
Systemic disease status at time of CNSide	
Active	16 (55.2)
Stable	13 (44.8)
LMD-directed therapy prior to CNSide	
Yes	13 (44.8)
No	16 (55.2)
LMD-directed therapy after CNSide	
Yes	26 (89.7)
No	3 (10.3)

Most patients had radiographic evidence of LMD (26 with and 3 without). Median CSF-TC density was 67.8 TCs/mL (IQR, 4.7–639 TCs/mL). A narrow majority of patients had positive CSF cytology (55.2%) and CSF-TC density was significantly higher in patients with positive cytology (median 527.9 vs 4.5 TCs/mL, $P = .18x10^{-5}$) (Fig. 1). Median follow-up after CNSide testing was 159 days (IQR, 89–257; range, 5-593), by which point 19 patients died related to disease, 9 remained alive, and 1 was presumed lost to follow-up.

Survival of 29 patients with CSF-TCs

Median overall survival from time of CSF-TC detection was 176 days (IQR, 89–481), 6-month survival was 47% (95% CI, 32–70%), and 1-year survival was 27% (95% CI, 13–57%). On univariable survival analysis, CSF-TC density was significantly associated with survival (HR = 1.39; 95% CI, 1.01–1.90; $P = .04$). Exploratory univariable analysis, not corrected for multiple comparisons, suggested a survival association for KPS, cytology, CSF glucose, and systemic disease status (Table 2). On multivariable survival regression, the HR for CSF-TC density remained significant and was relatively unchanged from

univariable analysis (HR = 1.50; 95% CI, 1.02–2.21; *P* = .04). Unlike on univariable analysis, age was significantly associated with survival (Table 3).

Table 2
Hazard ratios for survival on univariate Cox proportional hazards modeling. Cytology, protein, glucose, and CNSide were performed on CSF acquired from the same procedure. Abbreviations: CSF, cerebrospinal fluid; KPS, Karnofsky performance status; LMD, leptomeningeal disease; TC, tumor cells.

Variable (Comparison)	Hazard ratio (95% CI)	P-value
Age	0.98 (0.95–1.02)	.37
Primary cancer (Breast)	-	-
Lung	1.39 (0.52–3.72)	.51
Other	1.35 (0.28–6.45)	.71
Time from LMD diagnosis to CNSide	1.00 (1.00–1.00)	.12
KPS at CNSide (≥80)	4.20 (1.58–11.1)	.0040
Cytology (Negative)	4.30 (1.32-14.0)	.015
CSF protein, log(mg/dL)	4.32 (0.86–21.7)	.075
CSF glucose, mg/dL	0.96 (0.93–0.98)	.0029
CNSide TC density, log(TCs/mL)	1.39 (1.01–1.90)	.040
Systemic disease status at time of CNSide (Stable)	3.24 (1.16–9.06)	.025
LMD-directed therapy prior to CNSide (No)	0.64 (0.25–1.63)	.35
LMD-directed therapy after CNSide (No)	0.85 (0.18–3.75)	.80

Table 3
Hazard ratios for survival on multivariate Cox proportional hazards modeling. Abbreviations: CSF, cerebrospinal fluid; KPS, Karnofsky performance status; TC, tumor cells.

Variable (Comparison)	Hazard ratio (95% CI)	P-value
Age	0.94 (0.89–0.99)	.043
KPS at CNSide (≥80)	17.9 (3.38–94.4)	.00069
Concurrent CSF protein, log(mg/dL)	0.28 (0.04–2.11)	.22
CNSide CSF-TC density, log(TCs/mL)	1.50 (1.02–2.21)	.041
Systemic disease status at time of CNSide (Stable)	3.11 (1.02–9.47)	.046

An optimal cutpoint for survival by CSF-TC density was determined to be ≤19.34 (low risk) vs > 19.34 TCs/mL (high risk) (Fig. 2). Notably, no patients had a CSF-TC density between 19.34 and 67.78 TCs/mL.

In the low-risk group, 6 of 14 patients died during the study period, with a median survival of 481 days (IQR, 263-not reached). In the high-risk group, 13 of 15 patients died during the study period with a median survival of 89 days (IQR, 24–159). Using this CSF-TC density cutpoint, the time-dependent sensitivity and specificity for survival were 76% and 67% at 6 months and 65% and 67% at 1 year, respectively.

Discussion

Current LMD risk-stratification guidelines do not include an objective prognostic measure for patients with LMD [7, 8]. The findings of this retrospective cohort study suggest CSF-TC density from the CNSide assay has prognostic utility within an unselected, heterogenous patient population. The identified risk-stratification cutpoint of 19.34 TCs/mL approximates estimates from EpCAM-based assays, implying generalizability of study results [10, 15]. This cutpoint can potentially be incorporated into risk-stratification guidelines to assist medical decision making. Further, the high diagnostic sensitivity and specificity of CNSide CSF-TCs in this study reinforces the utility of this technique compared to cytology.

Limitations

The granularity of the study results is limited by sample size and patient heterogeneity, particularly regarding oncologic therapy diversity within the study population. No patients in the study cohort had a CSF-TC density value between 19.34 and 67.78 TCs/mL, creating uncertainty for how to approach patients with a CSF-TC density in this range. The proposed risk-stratification cutpoint has not been prospectively validated in the context of clinical decision making.

Conclusions

The results of this retrospective cohort study demonstrate the prognostic value of CSF-TC density from the CNSide assay. Integrating CSF-TC density into LMD patient risk-stratification models may help support treatment decision making.

Declarations

Conflict of Interest Statement for All Authors

ABB, LPT, JJG, MB, LMH, YDT, VV: no conflicts of interest.

BB: received salary and stock as an employee of Biocept.

TM: Research funding: Biomimetix, Denovo Biopharma, Biohaven pharmaceuticals, Chimerix, Servier, Novocure, Vigeo. Advisory board: Servier

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Data Availability Statement for this Work

The source data is available upon reasonable request to the corresponding author

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Author Contribution

A.B.B. and J.T.Y. wrote the original manuscript text. A.B.B. prepared Figs 1 and 2, performed statistical analysis, and prepared the tables. All authors reviewed the manuscript.

References

1. Barbour AB, Kotecha R, Lazarev S, Palmer JD, Robinson T, Yerramilli D, Yang JT (2023) Radiation Therapy in the Management of Leptomeningeal Disease From Solid Tumors. Adv Radiat Oncol. https://doi.org/10.1016/j.adro.2023.101377
2. Yang JT, Wijetunga NA, Pentsova E, Wolden S, Young RJ, Correa D, Zhang Z, Zheng J, Steckler A, Bucwinska W et al (2022) Randomized Phase II Trial of Proton Craniospinal Irradiation Versus Photon Involved-Field Radiotherapy for Patients With Solid Tumor Leptomeningeal Metastasis. J Clin Oncol JCO2201148. 10.1200/JCO.22.01148
3. Nayak L, Fleisher M, Gonzalez-Espinoza R, Lin O, Panageas K, Reiner A, Liu CM, Deangelis LM, Omuro A (2013) Rare cell capture technology for the diagnosis of leptomeningeal metastasis in solid tumors. Neurology 80:1598–1605 discussion 1603. 10.1212/WNL.0b013e31828f183f

4. Diaz M, Singh P, Kotchetkov IS, Skakodub A, Meng A, Tamer C, Young RJ, Reiner AS, Panageas KS, Ramanathan LV et al (2022) Quantitative assessment of circulating tumor cells in cerebrospinal fluid as a clinical tool to predict survival in leptomeningeal metastases. J Neurooncol 157:81–90. 10.1007/s11060-022-03949-1
5. Appel H, Odia Y, Saxena A, Roy M, Mohler A, Kotecha R, Hall MD, Ahluwalia MS, Mehta MP, Castaneda SA (2023) Evaluating the diagnostic performance of leptomeningeal diagnosis with CNSide compared to standard cytology. *J Clin Oncol 41*
6. Ghaferi AA, Schwartz TA, Pawlik TM (2021) STROBE Reporting Guidelines for Observational Studies. JAMA Surg 156:577–578. 10.1001/jamasurg.2021.0528
7. Le Rhun E, Weller M, Brandsma D, Van den Bent M, de Azambuja E, Henriksson R, Boulanger T, Peters S, Watts C, Wick W et al (2017) EANO-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of patients with leptomeningeal metastasis from solid tumours. Ann Oncol 28:iv84–iv99. 10.1093/annonc/mdx221
8. NCCN (2023) National Comprehensive Cancer Network: Central Nervous System Cancers (Version 1.2023)
9. Hyun JW, Jeong IH, Joung A, Cho HJ, Kim SH, Kim HJ (2016) Leptomeningeal metastasis: Clinical experience of 519 cases. Eur J Cancer 56:107–114. 10.1016/j.ejca.2015.12.021
10. Wijetunga NA, Boire A, Young RJ, Yamada Y, Wolden S, Yu H, Kris M, Seidman A, Betof-Warner A, Diaz M et al (2021) Quantitative cerebrospinal fluid circulating tumor cells are a potential biomarker of response for proton craniospinal irradiation for leptomeningeal metastasis. Neurooncol Adv 3:vdab181. 10.1093/noajnl/vdab181
11. Malani R, Bhatia A, Warner AB, Yang JT (2023) Leptomeningeal Carcinomatosis from Solid Tumor Malignancies: Treatment Strategies and Biomarkers. Semin Neurol 43:859–866. 10.1055/s-0043-1776996
12. Ogluszka M, Orzechowska M, Jedroszka D, Witas P, Bednarek AK (2019) Evaluate Cutpoints: Adaptable continuous data distribution system for determining survival in Kaplan-Meier estimator. Comput Methods Programs Biomed 177:133–139. 10.1016/j.cmpb.2019.05.023
13. Cantor AB, Shuster JJ (1994) Re - Dangers of Using Optimal Cutpoints in the Evaluation of Prognostic Factors. Jnci-J Natl Cancer I 86:1798–1799. 10.1093/jnci/86.23.1798-a
14. Heagerty PJ, Lumley T, Pepe MS (2000) Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 56:337–344. 10.1111/j.0006-341x.2000.00337.x
15. Chabot K, Osei-Gyening I, Estrera R, Yang J, Pentsova E, Boire A, Wilcox J (2023) EPID-04. EVOLVING SURVIVAL IN PATIENTS WITH LEPTOMENINGEAL METASTASES FROM SOLID TUMORS. Neuro Oncol 25:v115

Figures

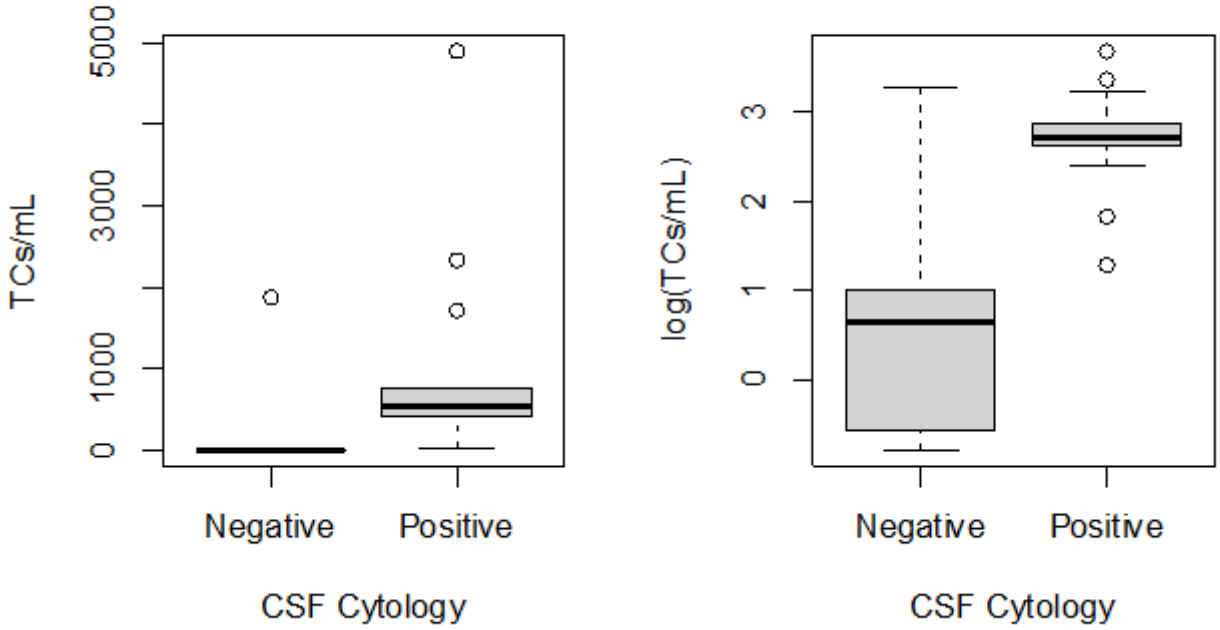


Figure 1

Boxplots of CSF cytology and CNSide tumor cell density. For 29 patients with positive CNSide testing, cerebrospinal fluid (CSF) cytology results are plotted against CNSide CSF tumor cell (TC) density and log-transformed CSF-TC density. CNSide and cytology were performed on CSF acquired from the same procedure.

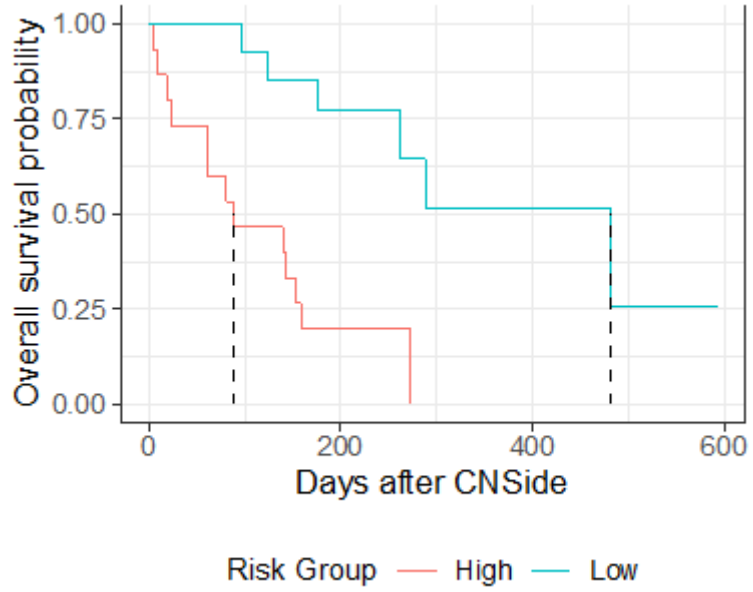


Figure 2

Kaplan-Meier survival by cerebrospinal fluid tumor cell (CSF-TC) density risk group. Kaplan-Meier survival plotted with 95% confidence intervals and dashed lines representing median survival for each group. Risk groups were defined by having £19.34 (low risk) vs >19.34 CSF-TCs/mL (high risk) on CNSide testing. Survival was estimated in days following CNSide testing of CSF.