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Hepcidin as an emerging predictor biomarker of leptomeningeal metastases in patients with metastatic breast cancer

Constance Delaby^{1,2*}, Anas Al Herk³, Christophe Hirtz¹, William Jacot^{4,6}, Maryline Laigre⁴, Stéphane Pouderoux⁴, Nicolas Pradeilles¹, Sylvain Lehmann¹ and Amélie Darlix^{4,5}

Abstract

Background Leptomeningeal metastatic disease (LMD) occurs in 5–19% of solid tumor cases, with breast cancer being a leading cause. Limited penetration of therapeutic agents into the CNS and increased patient survival may contribute to the rising incidence. Diagnosis relies on cerebrospinal fluid (CSF) cytology and MRI, but these have limited sensitivity, highlighting the need for improved biomarkers.

Patients and methods In this exploratory study, we examined CSF and serum levels of NFL, GFAP, Tau, and Hepcidin in metastatic breast cancer patients with and without LM included in a prospective study, to evaluate their potential as biomarkers for LMD diagnosis, prognosis, and treatment decision-making.

Results A total of 49 adult patients were included, among which 18 patients (LMD + group) had confirmed LMD based on CSF cytology and 31 were not confirmed as having LMD (LMD- group). In CSF, median concentrations of Hepcidin were 0.3 ng/mL and 1.4 ng/mL in LMD- and LMD + patients, respectively ($p < 0.01$). Using ROC analysis, the AUC yielded by CSF biomarkers to detect LMD was 0.909 for Hepcidin, 0.841 for proteinorachy, 0.722 for both NFL and GFAP and 0.703 for t-Tau ($p < 0.05$). The AUC yielded by serum Hepcidin to detect LMD was 0.514.

Conclusions Our findings indicate that CSF Hepcidin exhibits strong diagnostic potential for LMD detection, which could help clinicians in the early identification of this condition.

Keywords Leptomeningeal metastases, Fluid Biomarkers, Diagnosis

*Correspondence:

Constance Delaby

constance.delaby@inserm.fr

¹LBPC-PPC, Université de Montpellier, INM INSERM, IRMB CHU de Montpellier, Montpellier, France

²Sant Pau Memory Unit, Department of Neurology, Institut d'Investigacions Biomèdiques Sant Pau Hospital de Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

³Biometric Unit, Institut Régional du Cancer de Montpellier, University of Montpellier, Montpellier, France

⁴Department of Medical Oncology, Institut Régional du Cancer de Montpellier, University of Montpellier, Montpellier, France

⁵Institute of Functional Genomics IGF, University of Montpellier, CNRS, INSERM, Montpellier, France

⁶Institut Régional du Cancer de Montpellier, Montpellier University, INSERM U1194, Montpellier, France

Background

Metastases in the central nervous system (CNS), Such as intraparenchymal brain metastases (BM) and leptomeningeal metastatic disease (LMD), are the most frequently occurring intracranial cancers [1]

In patients with metastatic breast cancer, factors that predict BM include younger age, advanced tumor stage at the time of breast cancer diagnosis (such as larger tumor size and greater nodal involvement), higher histological grade, estrogen receptor (ER) negativity, and amplification of the human epidermal growth factor receptor 2 (HER2) [2]. Earlier research conducted by



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our group on breast cancer patients indicates a correlation between BM and serum levels of HER2 extracellular domain, matrix metalloproteinase 9, and Tau (a microtubule-associated protein primarily found in the CNS) [3, 4]. Furthermore, we have previously evaluated the association of various neuronal proteins serum levels with the presence of BM and prognosis in patients with metastatic breast cancer, and showed potential predictive role of serum glial fibrillary acidic protein (GFAP) [5] and Tau [3].

LMD from solid tumors is observed in 5% to 19% of cases, with breast cancer being a leading cause [6]. As with BM, the incidence of LMD appears to be rising, likely due to prolonged survival in patients with metastatic breast cancer and the limited penetration of therapeutic agents into the CNS. LMD should be suspected in patients presenting with headaches accompanied by cranial and/or spinal nerve involvement. To confirm the diagnosis of LMD, the primary tests include conventional cytology of CSF samples (considered the gold standard) and/or MRI of the brain and spinal cord. However, as these techniques have limited sensitivity, repeated CSF samples to reach a diagnosis are often needed. In this context, news circulating tools to improve diagnosis are needed [7–9]. Although improved prediction of patient outcomes could help guide treatment choices, the role of circulating biomarkers in this context also remains to be investigated [7, 8].

Neurofilament light chain (NfL) is a structural protein in the neuronal cytoskeleton, predominantly found in myelinated axons [10]. Increased NfL levels in CSF are commonly observed as a marker of white matter injury in conditions such as traumatic brain injury, multiple sclerosis, neurodegenerative diseases, and stroke [10]. Given the correlation between NfL levels in serum and CSF, serum NfL has been proposed as a potential indicator of brain damage.

GFAP is a key component of the glial cytoskeleton, particularly in astrocytes, making it a promising biomarker for glial cell damage [11, 12]. Elevated GFAP serum levels have been reported in various conditions such as Alzheimer's disease, multiple sclerosis and BM.

Tau protein is mainly expressed in the CNS by neurons and glial cells. Elevated levels of total tau (t-Tau) in CSF are observed in various contexts, including Alzheimer's disease, traumatic brain injury and Creutzfeldt-Jakob disease, thus limiting the specificity of this biomarker [13]. Blood t-Tau concentrations and CSF levels show poor correlation with each other, and blood t-Tau is better considered as a general biomarker for brain injury, with increased levels seen in various contexts such as brain concussion [14], hypoxemia [15], and BM [3].

Hepcidin is a small peptide that regulates systemic iron availability by inducing cellular iron retention and

limiting iron circulation [16]. Hepcidin plasma level rises in response to iron overload and inflammation, while it decreases with erythropoietic stimulation and anemia [16]. Although being mainly synthesized by the liver, it can also be produced locally in various organs (including the brain), where it could play important roles in maintaining local iron homeostasis [17].

Given their potential value as biomarkers for the diagnosis and monitoring of brain metastases (NfL, GFAP, and tau reflecting axonal, glial, and neuronal injury, and hepcidin reflecting iron metabolism regulation and inflammation), our study sought to determine whether CSF and/or serum levels of these markers were associated with the presence of LMD in patients with metastatic breast cancer.

We thus measured these biomarker levels in CSF and serum patients with and without LM and assessed whether one or more of these biomarkers could help for the diagnosis of LMD, but also for prognostication.

Material and methods

Population included

A prospective, monocentric study (NCT03252912; promoted by the Institut du Cancer de Montpellier, France, Clinical trial number: not applicable) was conducted in order to evaluate the clinical sensitivity and specificity of the detection of circulating tumor cells in the cerebrospinal fluid (CSF) for the diagnosis of LMD in metastatic breast cancer patients with a suspicion of LMD based on clinical and/or radiological signs. The results of the primary endpoint on circulating tumor cells have been reported elsewhere [9]. A total of 49 adult patients were included in this study after giving their written informed consent (see [9] for full description), among which 18 patients (LMD+ group) had confirmed LMD based on CSF cytology (gold-standard) and 31 were not confirmed as having LMD (LMD- group). Cytological diagnosis of CSF was based on one to three samples according to the current guidelines in patients with suspected LMD. If tumor cells were found by conventional cytology in the first CSF sample, LM diagnosis was established, and no additional lumbar puncture was performed. If not, a second (or third, if required) CSF sample was collected. Only patients with three negative CSF samples were considered as not having LMD.

CSF and blood collection

Besides being analyzed for circulating tumor cells detection with the CellSearch® technique, CSF samples obtained from lumbar punctures at the time of LMD suspicion were biobanked as part of the study protocol, to enable further translational studies. Blood samples were collected as well.

Serum and CSF biomarkers quantification

GFAP and NfL concentrations were measured in CSF and blood using Simoa technology [18] with the commercial Neurology 2-Plex B (GFAP, NF-light) ultrasensitive immunoassay kit (Quanterix, USA) on Simoa HD-X analyser. Serum samples were diluted 1:4 with the provided dilution buffer as described in the assay protocol to minimize matrix effect. The accuracy of the assay was ensured using kit provided Low- and High-quality control samples, reflecting intra-assay CVs% below 5.1% for both analytes. Hepcidin-25 (active isoform) concentration was determined in CSF and blood using the validated HS competitive-ELISA kit (DRG Diagnostics GmbH, Marburg, Germany, ref EIA-5782). Serum samples were diluted 1:10 in provided dilution buffer and assayed according to manufacturer instruction. Provided controls showed an intra-plate CV% of 5.7% (Supplementary Table 1).

Statistical analysis

Categorical variables were reported as: number of observations, frequency of each modality and number of missing data. They were compared using the Chi-square test or the Fisher's exact test. Continuous variables (including biomarkers CSF and serum levels) were described as number of observations, median, range and number of missing data, and compared using the Kruskal–Wallis test. To evaluate the biomarker accuracy to identify patients with LMD, the clinical sensitivity, specificity, negative predictive value, and positive predictive value were calculated for each biomarker independently, and for combinations. The receiver operating characteristic (ROC) curve and area under the curve (AUC) were determined, using the result of CSF cytology as the reference (LMD+ vs. LMD-). As there are no validated cutoff values for NfL, GFAP and Hepcidin in this setting, median values were used. Logistic regressions were used to study the variables associated with LMD presence. Multivariate analyses were performed using a backward stepwise procedure and including variables with P -value < 0.05 of the univariate analyses while keeping the two variables (age and number of metastatic sites) irrespective of their P -values. Odd ratios (ORs) with their 95% confidence intervals (CIs) were calculated. As an exploratory endpoint, survival was analyzed in the LMD+ group only. Overall survival (OS) was defined as the time interval between the date of serum/CSF sample collection and the date of death from any cause, and neurological degradation-free survival as the time interval between the date of serum/CSF sample collection and the first documented neurological degradation. Patients alive without events were censored at the date of the last follow-up (cutoff date for the analysis: 10 July 2019). Survival distributions were estimated according to the Kaplan–Meier method,

presented as medians with their 95% CI, and compared to the log-rank test. Multivariate analyses were performed using the Cox's proportional hazards regression model and a backward stepwise procedure and including variables with P -value < 0.05 of the univariate analyses while keeping the two variables (age and number of metastatic sites) irrespective of their P -values. Hazard ratios (HRs) with their 95% CI were calculated.

Results

Demographic description of patient

A total of 49 eligible patients (47 women and 2 men) were enrolled between January 2017 and January 2020 (Table 1). The most common histological subtype was ductal carcinoma (77.5%). Molecular classification of breast cancer was as follows: HER2-/HR+ (67.3%), HER2+ (20.4%) and triple-negative (12.2%). At CSF sampling, the median age was 63 years (range: 32–77) and 79.6% of patients had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 . 93.9% of patients had already developed metastatic disease (median number of metastatic sites 3, range 1–6) and 34.8% had BM. LMD suspicion occurred after a median of 78.1 months (range: 2.2–316.2) following breast cancer diagnosis and 32.8 months (range: 0.6–217.7) after metastatic disease diagnosis. LMD was suspected based on clinical symptoms in 89.8% of cases, gait disturbances, nausea, cranial nerve involvement, headache and fatigue being the most frequent symptoms. Based on CSF analysis with conventional cytology, 18 patients were diagnosed with LMD (LMD+ group) while 31 were not (LMD- group).

Biomarkers assessment in CSF and serum according to the presence of LMD

Biomarkers were evaluated according to the absence (LMD-) or presence (LMD+) of LMD, Table 2. In the CSF, median concentrations of Hepcidin were 0.3 ng/mL and 1.4 ng/mL in LMD- and LMD+ respectively ($p < 0.01$). The median levels of NfL in the CSF were 1.9 pg/mL and 3.8 pg/mL ($p = 0.02$), median levels of CSF GFAP were 11.4 pg/mL and 22.5 pg/mL ($p = 0.01$) and median levels of t-Tau were 148.5 pg/mL and 263.5 pg/mL ($p = 0.02$) in LMD- and LMD+, respectively, Table 2. In the serum, median concentrations of Hepcidin were 22.6 ng/mL and 22.8 ng/mL in LMD- and LMD+, respectively ($p = 0.88$). In the serum, median levels of NfL were 52.1 pg/mL and 97.2 pg/mL ($p = 0.14$) and GFAP median levels were 168.7 pg/mL and 304.5 pg/mL in LMD- and LMD+, respectively ($p = 0.08$). Of note, no significant difference in serum and CSF levels were observed according to the time of the day (before noon and after noon) the CSF or blood was collected (Supplementary Table 2).

Table 1 Demographic and clinical data, TNM stage, biological status (HER, ER, PR), histological grade, presence of metastases, number of metastatic sites, brain metastasis and performance status of LM- and LM+ patients

	LMD- (n = 31)	LMD + (n = 18)	Total (n = 49)	p value
Age at diagnosis				0.20 ¹
N	31	18	49	
Min—Max	30.1—76.2	30.3—69.6	30.1—76.2	
Sex				0.27 ²
Man	2 (6.5%)	0 (0.0%)	2 (4.1%)	
Woman	29 (93.5%)	18 (100.0%)	47 (95.9%)	
TNM: Stage T				0.05 ²
T0	0 (0.0%)	1 (6.2%)	1 (2.5%)	
T1	7 (29.2%)	5 (31.2%)	12 (30.0%)	
T2	11 (45.8%)	3 (18.8%)	14 (35.0%)	
T3	6 (25.0%)	3 (18.8%)	9 (22.5%)	
T4	0 (0.0%)	4 (25.0%)	4 (10.0%)	
Missing	7	2	9	
TNM: Stage N				0.44 ²
N0	8 (29.6%)	3 (20.0%)	11 (26.2%)	
N1	10 (37.0%)	8 (53.3%)	18 (42.9%)	
N2	6 (22.2%)	4 (26.7%)	10 (23.8%)	
N3	3 (11.1%)	0 (0.0%)	3 (7.1%)	
Missing	4	3	7	
TNM: Stage M				0.46 ²
M-	19 (79.2%)	11 (68.8%)	30 (75.0%)	
M +	5 (20.8%)	5 (31.2%)	10 (25.0%)	
Missing	7	2	9	
Biological group				0.86 ²
Triple negative	2 (6.5%)	3 (16.7%)	5 (10.2%)	
HER2- ER + PR-	7 (22.6%)	4 (22.2%)	11 (22.4%)	
HER2- ER + PR +	16 (51.6%)	8 (44.4%)	24 (49.0%)	
HER2 + ER- PR-	2 (6.5%)	1 (5.6%)	3 (6.1%)	
HER2 + ER + PR-	1 (3.2%)	0 (0.0%)	1 (2.0%)	
HER2 + ER + PR +	3 (9.7%)	2 (11.1%)	5 (10.2%)	
Histological Grade				0.27 ²
1	1 (3.2%)	2 (11.1%)	3 (6.1%)	
2	14 (45.2%)	11 (61.1%)	25 (51.0%)	
3	15 (48.4%)	4 (22.2%)	19 (38.8%)	
X	1 (3.2%)	1 (5.6%)	2 (4.1%)	
Histology				< 0.01 ²
No specific type	26 (83.9%)	10 (55.6%)	36 (73.5%)	
Lobular	2 (6.5%)	8 (44.4%)	10 (20.4%)	
No specific type + other	2 (6.5%)	0 (0.0%)	2 (4.1%)	
Other	1 (3.2%)	0 (0.0%)	1 (2.0%)	
Missing	1	0	1	
Presence of metastases				
No	3 (9.7%)	0 (0.0%)	3 (6.1%)	
Yes	28 (90.3%)	18 (100.0%)	46 (93.9%)	
Number of metastatic sites				0.28 ¹
N	31	18	49	
Mean (SD)	2.7 (1.9)	3.1 (1.3)	2.8 (1.7)	
Median (Q1, Q3)	2.0 (1.0, 4.0)	3.0 (2.2, 4.0)	3.0 (1.0, 4.0)	
Min—Max	0.0—6.0	1.0—5.0	0.0—6.0	
Brain metastases				0.64 ²
No	19 (67.9%)	11 (61.1%)	30 (65.2%)	
Yes	9 (32.1%)	7 (38.9%)	16 (34.8%)	

Table 1 (continued)

	LMD- (<i>n</i> = 31)	LMD+ (<i>n</i> = 18)	Total (<i>n</i> = 49)	<i>p</i> value
Performance status				0.09 ²
0	5 (16.1%)	3 (16.7%)	8 (16.3%)	
1	14 (45.2%)	4 (22.2%)	18 (36.7%)	
2	9 (29.0%)	4 (22.2%)	13 (26.5%)	
3	3 (9.7%)	7 (38.9%)	10 (20.4%)	

¹Kruskal-Wallis rank sum test²Pearson's Chi-squared test

*Squamous cell carcinoma, Medullary cancer and Intra-canalicular + Mucinous Colloid

Diagnostic accuracy of CSF biomarkers for the presence of LMD

We performed ROC analysis to assess the diagnostic accuracy of each CSF biomarker to classify participants as positive (LMD+) or negative (LMD-) for the presence of LMD. The AUC yielded by CSF biomarkers to detect LMD were 0.722 for both NfL and GFAP and 0.909 for Hepcidin ($p < 0.05$), Table 3 and Fig. 1. The combination of these biomarkers resulted in AUC similar to Hepcidin alone, with a p value of 0.915, Table 3 and Fig. 1. We also assessed AUC values for CSF t-Tau and proteinorachy (Table 3), which were 0.703 and 0.841, respectively ($p = 0.09$).

Diagnostic accuracy of serum biomarkers for the presence of LMD

We next calculated the accuracy of each individual biomarker in serum to discriminate participants classified as LMD- from those LMD+. As shown in Table 3 and Fig. 2, the AUC yielded by serum biomarkers to detect LMD were 0.630, 0.654 and 0.514 for NfL, GFAP and Hepcidin, respectively. The combination of these biomarkers resulted in slightly increased AUC value (although not specifically different from the AUC obtained with GFAP alone), reaching 0.671 ($p = 0.89$), Table 3 and Fig. 2.

Correlation of CSF and serum biomarkers with overall survival (OS)

In univariate analysis (Table 4), high CSF levels of Hepcidin, GFAP and t-Tau were associated with shorter OS in the LMD+ group ($p = 0.026$, $p = 0.05$, and $p = 0.046$, respectively), while no association with OS was detected for CSF NfL ($p = 0.129$). Multivariate analysis showed no significant performance of CSF GFAP ($p = 0.132$) to predict OS, neither did CSF Hepcidin ($p = 0.065$) or t-Tau ($p = 0.196$). In serum, GFAP levels were associated with decreased OS in univariate analysis, $p = 0.018$ (Table 4). In multivariate analysis, none of the serum biomarkers tested was informative of OS.

Correlation of CSF and serum biomarkers with neurological degradation-free survival

In univariate analysis (Table 5), only high CSF levels of GFAP were associated with shorter neurological degradation-free survival in the LMD+ group ($p = 0.045$), while no association was detected for CSF Hepcidin ($p = 0.072$), CSF NfL ($p = 0.175$), and CSF t-Tau ($p = 0.383$). In serum, none of the biomarker tested was informative for prognosis of neurological degradation-free survival (Table 5).

Discussion

LMD is identified in 5% to 19% of solid tumor cases, with breast cancer being a major contributor [6]. LMD is diagnosed by detecting tumor cells in CSF using conventional cytology, considered as the gold standard. So far, the role of circulating biomarkers in this context has remained uncertain although some candidates may be promising, such as neural biomarkers. In particular, Tau, GFAP, and NfL serve as brain protein biomarkers with the potential to enhance diagnostic precision, monitor disease progression, assess prognosis, and evaluate treatment outcomes in various neurological diseases. Their release into CSF and blood reflects the severity of neuronal and astrocytic damage in a range of neurological conditions, including stroke, traumatic brain injury, multiple sclerosis, neurodegenerative dementias, and Parkinson's disease [19]. Moreover, Tau, GFAP and NfL are gaining recognition as biomarkers in oncology, particularly concerning their roles in brain tumors [20] and chemotherapy-induced cognitive impairment [21]. Elevated levels of these proteins in the blood have in particular been associated with brain injuries and tumors, suggesting their potential as non-invasive biomarkers for detecting central nervous system malignancies [20]. Importantly, we have previously described the informativeness of Tau, GFAP and NfL in the diagnosis or prognosis of BM [3, 5].

On the other hand, emerging research suggests the potential role of Lipocalin-2 (LCN2) as an informative diagnosis biomarker of LMD in the context of brain metastasis [22, 23]. Lipocalins are a family of small, secreted proteins involved in various physiological processes, including immune responses and transport of small hydrophobic molecules like lipids and

Table 2 CSF (Hepcidin, NfL, GFAP, t-Tau, proteins) and serum (Hepcidin, NfL, GFAP) biomarkers' quantification in LM- and LM+ patients

Biomarkers	LM—(n = 31)	LM+ (n = 18)	Total (n = 49)	p value
CSF				
Hepcidin (ng/mL)				< 0.01 ¹
N	28	18	46	
Missing	3	0	3	
Mean (SD)	0.3 (0.3)	4.1 (9.3)	1.8 (6.0)	
Median (Q1, Q3)	0.3 (0.1, 0.4)	1.4 (0.7, 2.2)	0.4 (0.2, 1.1)	
Min—Max	0.0—1.6	0.2—40.6	0.0—40.6	
NfL (pg/mL)				0.02 ¹
N	29	15	44	
Missing	2	3	5	
Mean (SD)	2.6 (2.5)	5.4 (4.4)	3.6 (3.5)	
Median (Q1, Q3)	1.9 (0.6, 4.2)	3.8 (1.9, 10.3)	2.1 (0.7, 5.1)	
Min—Max	0.3—8.7	0.5—13.4	0.3—13.4	
GFAP (pg/mL)				0.01 ¹
N	31	18	49	
Missing	0	0	0	
Mean (SD)	14.0 (11.6)	27.9 (35.3)	19.1 (23.9)	
Median (Q1, Q3)	11.4 (6.7, 15.0)	22.5 (10.9, 25.8)	12.5 (8.4, 22.3)	
Min—Max	2.9—54.3	5.9—163.6	2.9—163.6	
T-TAU (pg/mL)				0.02 ¹
N	30	18	48	
Missing	1	0	1	
Mean (SD)	212.0 (214.8)	413.3 (461.5)	287.5 (339.4)	
Median (Q1, Q3)	148.5 (110.8, 234.2)	263.5 (163.2, 414.2)	166.5 (126.2, 313.8)	
Min—Max	70.0—1252.0	76.0—2000.0	70.0—2000.0	
Protein (g/L)				< 0.01 ¹
N	31	17	48	
Missing	0	1	1	
Mean (SD)	0.5 (0.3)	2.2 (2.4)	1.1 (1.6)	
Median (Q1, Q3)	0.4 (0.3, 0.6)	1.4 (0.6, 2.4)	0.5 (0.3, 0.9)	
Min—Max	0.2—2.0	0.3—8.4	0.2—8.4	
SERUM				
Hepcidin (ng/mL)				0.87 ¹
N	25	18	43	
Missing	6	0	6	
Mean (SD)	27.1 (20.8)	33.4 (30.6)	29.8 (25.2)	
Median (Q1, Q3)	22.6 (13.7, 29.9)	22.8 (9.7, 44.3)	22.6 (13.3, 37.1)	
Min—Max	5.4—90.9	5.5—107.2	5.4—107.2	
NfL (pg/mL)				0.14 ¹
N	30	18	48	
Missing	1	0	1	
Mean (SD)	112.8 (168.9)	118.0 (89.3)	114.8 (143.1)	
Median (Q1, Q3)	52.1 (27.2, 132.4)	97.2 (53.1, 165.4)	63.5 (32.9, 139.8)	
Min—Max	9.7—852.7	18.6—341.4	9.7—852.7	
GFAP (pg/mL)				0.08 ¹
N	30	18	48	
Missing	1	0	1	
Mean (SD)	245.0 (230.9)	392.8 (299.0)	300.4 (265.5)	
Median (Q1, Q3)	168.7 (112.8, 227.7)	304.5 (163.0, 545.0)	190.1 (121.1, 415.6)	
Min—Max	54.1—1126.4	76.7—1144.0	54.1—1144.0	

¹Kruskal–Wallis rank sum test

Table 3 AUC, sensibility and specificity of biomarkers measured in the CSF (t-Tau, proteins, Hepcidin, NfL, GFAP and association of [Hepcidin, NfL, GFAP]) and the serum (Hepcidin, NfL, GFAP and association of [Hepcidin, NfL, GFAP]) between LM- and LM+ patients

Fluid	Biomarker	AUC (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)
CSF	t-Tau	70.3 [53.7;86.9]	72.2 [38.9;94.4]	70 [53.3;96.7]
	Protein	84.1 [71.9;96.2]	70.6 [47.1;94.1]	87.1 [58.1;100]
	Hepcidin	90.9 [82.2;99.6]	88.9 [72.2;100]	85.7 [71.4;100]
	NfL	72.2 [56.3;88]	93.3 [33.3;100]	44.8 [34.5;100]
	GFAP	72.2 [57.1;87.4]	55.6 [38.9;100]	90.3 [38.7;96.8]
	Hepcidin-NfL-GFAP	91.5 [83.2;99.9]	93.3 [66.7;100]	76.9 [61.5;100]
Serum	Hepcidin	51.4 [32.7;70.2]	27.8 [11.1;100]	88 [8;100]
	NfL	63.0 [46.9;79.1]	77.8 [38.9;100]	50 [20;93.3]
	GFAP	65.4 [48.3;82.4]	61.1 [33.3;94.4]	76.7 [40;96.7]
	Hepcidin-NfL-GFAP	67.1 [49.2;85]	61.1 [33.3;88.9]	80 [56;96]

hormones. The co-regulation of LCN2 and hepcidin has been observed in some inflammatory and cancer contexts, including in the CNS [24]. Considering the roles of lipocalin-2 (NGAL) and hepcidin in immunity and iron metabolism, particularly in restricting iron availability, the NGAL–hepcidin axis may foster an iron-enriched intracellular environment that facilitates tumor growth. Further exploration of this pathway as a source of potential biomarkers and therapeutic targets in brain injury or metastases could therefore be highly informative.

In the present work, we show that CSF Hepcidin is highly informative of the presence of LMD in metastatic breast cancer patients, with an AUC value reaching 0.909 (95% CI: [0.822–0.996]). Furthermore, no significant increase of AUC was observed when combining CSF Hepcidin with the other biomarkers tested (NfL, GFAP) through linear regression, the value reaching 0.915; 95%CI: [0.832–0.999]). Additionally, we evaluated the potential benefit of adding CSF t-Tau protein or proteinorachy to improve the performance of CSF Hepcidin, but neither of these additions enhanced the performance achieved by Hepcidin on its own ($p=0.98$ and 0.93 , respectively, *not shown*). Interestingly, serum Hepcidin remains poorly informative of LMD, including when combined with serum NfL and GFAP (AUC = 0.514; 95% CI: [0.327–0.702] and AUC = 0.671; 95% CI: [0.492–0.850], respectively).

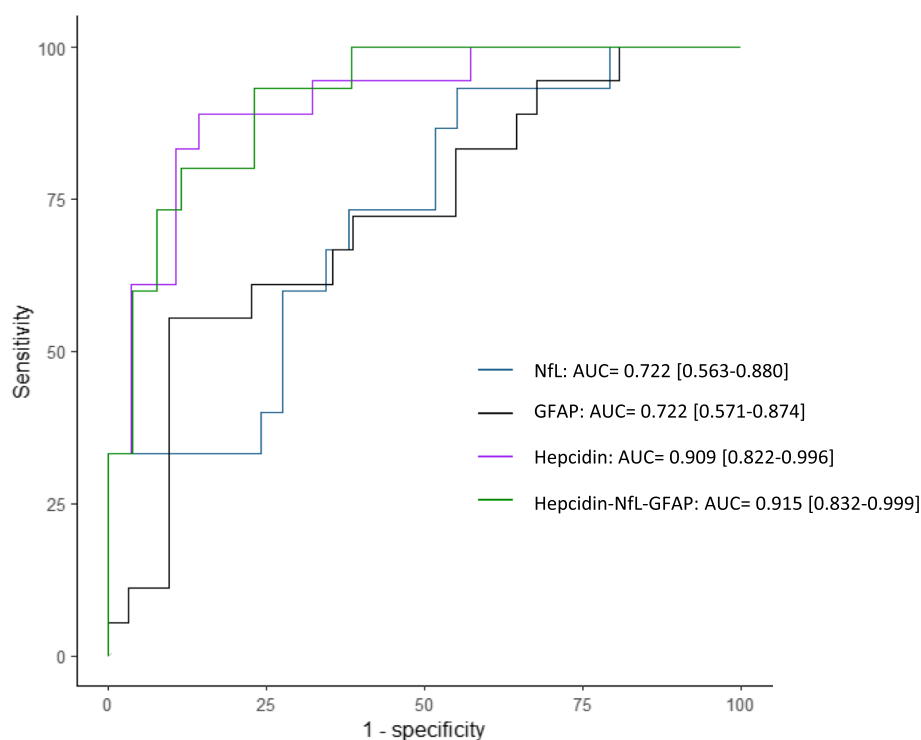


Fig. 1 Evaluation of CSF biomarkers' informativity for LM diagnosis: the graph compares receiving operator characteristics (ROC) analysis adjusted for age and sex of NfL (blue line), GFAP (black line), Hepcidin (purple line) and regression model combining all biomarkers (green line) between LM+ and LM- patients

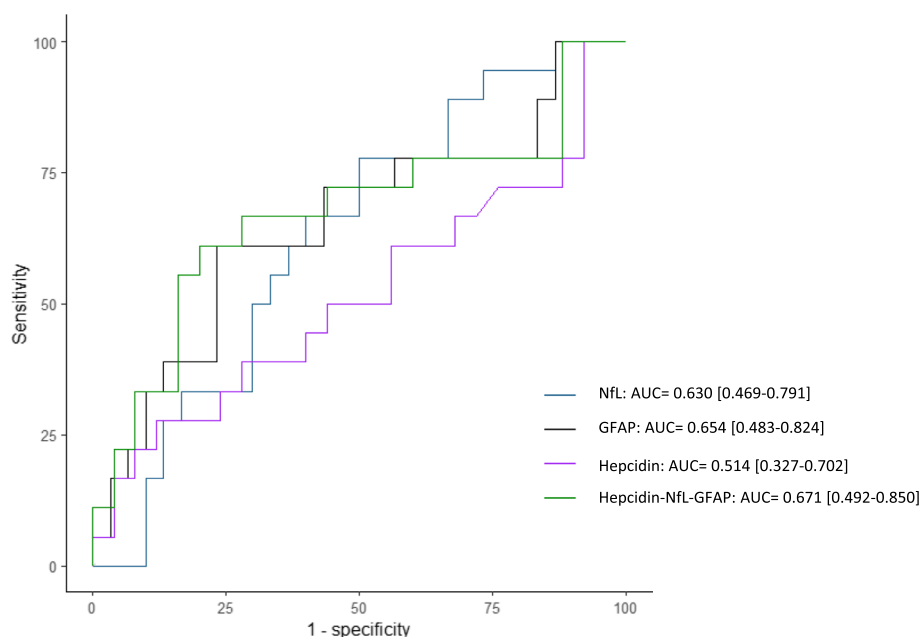


Fig. 2 Evaluation of serum biomarkers' informativity for LM diagnosis: the graph compares receiving operator characteristics (ROC) analysis adjusted for age and sex of NFL (blue line), GFAP (black line), Hepcidin (purple line) and regression model combining all biomarkers (green line) between LM+ and LM- patients

Table 4 Biomarkers and overall survival: univariate and multivariate cox models of hazard ratio are presented for age, number of metastatic sites, CSF (Hepcidin, NFL, GFAP, t-Tau, proteinorachy) and serum (Hepcidin, NFL, GFAP) biomarkers

Parameter	Univariate cox model: Hazard ratio (95% CI): <i>N</i> = 18, Events = 15	<i>P</i> value (univariate cox model)	Multi-variate cox model: Hazard ratio (95% CI): <i>N</i> = 18, Events = 15	<i>P</i> value (multivariate cox model)
Age	1.01 [0.95,1.07]	0.834	1.03 [0.95,1.12]	0.450
Number of metastatic sites	0.7 [0.45,1.09]	0.119	0.81 [0.43,1.52]	0.514
CSF Biomarker				
Hepcidin	1.09 [1.01,1.17]	0.026	1.11 [0.99,1.23]	0.065
NfL	0.89 [0.77,1.03]	0.129		
GFAP	1.03 [1,1.06]	0.051	1.03 [0.99,1.06]	0.132
T-Tau	1 [1,1]	0.046	1 [1,1]	0.196
CSF Protein	1.02 [0.8,1.28]	0.892		
Serum Biomarker				
Hepcidin	1.02 [1,1.04]	0.102		
NfL	1 [0.99,1]	0.252		
GFAP	1 [1,1]	0.018		

The biomarkers analyzed showed limited value in predicting neurological degradation-free survival, with CSF GFAP demonstrating predictive significance only in univariate COX models ($p=0.045$). However, one of the limits of this study relies on the relatively small number of patients, particularly in the group with confirmed LMD, which may limit the accuracy and reliability of the results. In addition, no longitudinal data was available. In univariate analysis, high CSF hepcidin and GFAP levels were associated with shorter OS, and multivariate analysis revealed a trend for CSF hepcidin ($p=0.059$) and confirmed the significant association for CSF GFAP

($p=0.043$). In serum, GFAP was linked to reduced OS in univariate analysis, but no serum biomarkers were significant in multivariate analysis.

This study suffers from some limitations. First, while our results are encouraging, this preliminary single-center study included a limited number of patients, inducing a limited statistical power. While our results demonstrate interesting results in terms of diagnostic value, results regarding prognosis are limited due to sample size and must be tested in larger cohorts. In addition, the lack of longitudinal sampling precludes the kinetic analysis of the diagnostic, prognostic and predictive value of

Table 5 Biomarkers and neurological degradation-free survival: univariate and multivariate cox models of hazard ratio are presented for age, number of metastatic sites, CSF (Hepcidin, NfL, GFAP, t-Tau, proteinorachy) and serum (Hepcidin, NfL, GFAP) biomarkers

Parameter	Univariate cox model: Hazard ratio (95% CI): <i>N</i> = 18, Events = 18	<i>P</i> value (univariate cox model)	Multi-variate cox model: Hazard ratio (95% CI): <i>N</i> = 18, Events = 18	<i>P</i> value (multivariate cox model)
Age	1.01 [0.95,1.07]	0.749	1.04 [0.97,1.12]	0.272
Number of metastatic sites	0.63 [0.4,1]	0.048	0.61 [0.35,1.04]	0.069
CSF Biomarker				
Hepcidin	1.05 [1,1.11]	0.072		
NfL	0.91 [0.8,1.04]	0.175		
GFAP	1.03 [1,1.06]	0.045	1.02 [1,1.05]	0.088
T-TAU	1 [1,1]	0.383		
CSF Protein	0.96 [0.77,1.21]	0.756		
Serum biomarker				
Hepcidin	1.01 [0.99,1.03]	0.305		
NfL	1 [0.99,1]	0.686		
GFAP	1 [1,1]	0.084		

this biomarker. Such hypothesis must be tested in larger multicenter studies, including patients treated for their LMD evolution, in order to evaluate if the kinetics of the biomarker is able to predict response to therapy. Lastly, these larger cohorts would enable the establishment and validation of markers thresholds to be used in the clinics.

Conclusion

As better prediction of patient outcomes could guide treatment decisions, we aimed in this study to assess the potential role of NfL, GFAP, Tau and Hepcidin as diagnostic and/or prognostic biomarkers of LMD in metastatic breast cancer patients. Our results show that CSF Hepcidin has high diagnostic potential for the detection of LMD, which may enable earlier and more accurate detection.

Further research in particular on larger cohorts of patients with suspected LMD is needed to validate the clinical application of Hepcidin for the diagnosis of LMD, and to explore its value for the prognostication and follow-up of patients, in order to improve their management.

Abbreviations

AUC	Area under the curve
BM	Brain metastases
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
ER	Estrogen receptor
GFAP	Glial fibrillary acidic protein
HR	Hazard ratio
LMD	Leptomeningeal metastatic disease
LOD	Limit of detection
LOQ	Limit of quantification
MRI	Magnetic resonance imaging
NfL	Neurofilament light chain
OR	Odd ratio
ROC	Receiver Operating Characteristic

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-025-15124-6>.

Supplementary Material 1: Supplemental Table 1. Analytical performance of biomarkers' assay: The table provides values of limit of detection (LOD), limit of quantification (LOQ) and intra-assay variability (% CV) for GFAP, NfL and Hepcidin quantification. Supplemental Table 2. Biomarker concentrations in CSF and serum according to the time of lumbar puncture during the day (before noon vs. after noon).

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Author's contributions

—C.D, S. L, C.H, A.D, W.J: Conceptualization; Methodology; Formal Analysis; Writing; Original draft preparation; Project administration—A.A: Methodology; Formal Analysis; Review & Editing manuscript—M.L, S. P, W. J: patients recruitment; Review & Editing manuscript—N. P: Investigation; Review & Editing manuscript.

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Data availability

Data available upon reasonable request to authors.

Declarations

Ethical approval and consent to participate

All experiments were performed in accordance with the Good Clinical Practice (GCP) guidelines as defined by the International Conference on Harmonization (ICH), and the French regulations in force (i.e., the French Public Health Law N. 2004–806 of August 9, 2004; Loi n. 2004- 806 du 9 août 2004 relative à la politique de santé publique), the Bioethical Laws, the declaration of Helsinki (Appendix 9) and the Informatics and Freedom Law. This study is registered at ClinicalTrials.gov (<http://clinicaltrials.gov>: Identifier NCT03252912). It was approved by the Ethics Committee (CPP SUD MEDITERRANEE 13/07/2016, n°2016–05-03 bis). The patient's written informed consent was obtained before collecting any personal data.

Consent for publication

All contributing authors approve the submission and publication of this manuscript.

Competing interests

- AD, SL, CH, SP, ML, NP, AA and CD declare no conflict of interest related to this study.

- WJ has received fees (Astra Zeneca, Eisai, BMS, Lilly France, Daiichi Sankyo, MSD, Novartis, Pfizer, Roche, Seagen), support for attending meetings/travel (Astra Zeneca, Novartis, Chugai Pharma, Pfizer, Eisai, Pierre Fabre, Glaxo Smithkline, Roche, Lilly France, Sanofi Aventis) and participated on a Data Safety Monitoring Board or Advisory Board (Astra Zeneca, Eisai, BMS, Lilly France, Daiichi Sankyo, MSD, Novartis, Pfizer, Roche, Seagen, Gilead).

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