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Intrathecal synthesis of tumor markers is a highly sensitive test in the diagnosis of leptomeningeal metastasis from solid cancers

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Abstract

Background: Identification of neoplastic cells in cerebrospinal fluid (CSF) by cytological analysis is the key diagnostic feature of leptomeningeal metastasis (LM). Because of the lack of sensitivity of this test, considerable efforts have been made to identify alternative diagnostic markers. Data from the literature suggest that measurement of tumor markers (TM) in CSF may be helpful for improving the diagnosis.
Methods: We analyzed the concentrations of the TM carcinoembryonic antigen (CEA), CA15.3, CA125 and CA19.9 in both CSF and serum from 18 patients with neoplastic meningitis diagnosed by CSF cytology. We also performed these same measurements in 50 patients affected by other neurological diseases (OND) in order to evaluate putative intrathecal synthesis. In addition, CSF and serum concentrations of the proangiogenic factor VEGF (vascular endothelial growth factor) were evaluated.
Results: All LM patients showed intrathecal synthesis for at least one TM. In one patient, a negative CSF cytology after treatment paralleled normalization of tumor marker synthesis. None of the OND patients displayed intrathecal TM synthesis. The VEGF Index (CSF/serum VEGF relative to CSF/serum albumin ratios) was significantly higher in LM patients compared with the control group. However, significant overlap between LM patients and values seen in those with OND was observed.
Conclusions: Evaluation of intrathecal TM synthesis is a specific, sensitive, reliable, and reproducible diagnostic tool, and is useful to support diagnosis of carcinomatous meningitis.
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Keywords: cerebrospinal fluid (CSF); diagnosis; leptomeningel metastasis; tumor markers.

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Introduction

Leptomeningeal metastasis (LM) is a complication occurring in oncological patients when tumor cells spread into the subarachnoid space and cerebrospinal fluid (CSF) compartment. According to autopsy studies, LM occurs in 5%–8% of cancer cases. However, the incidence is increasing and is probably due to more effective therapies and better diagnostic tools (1, 2). Although LM is a late complication in most patients, in 5%–10% it is the first manifestation of cancer (3). Prognosis is generally poor, with a mean survival time of 3.5–6 months (4). However, some patients survive longer than 12 months, particularly those with breast cancer (5). Early diagnosis is crucial to begin aggressive therapy and to prevent progressive neurological deterioration.

The ''gold standard'' for the diagnosis of LM is positive CSF cytological analysis for tumor cells. Unfortunately, this test has low-sensitivity, 50%–60% at initial lumbar puncture, which can be improved to 80% with repeated sampling of CSF (6, 7).

The tumor markers (TM) carcinoembryonic antigen (CEA), CA15.3, CA125 and CA19.9 are glycoproteins expressed on various cell types, but produced in higher concentrations by cancer cells. Higher TM levels in serum are not considered specific for malignancies, since they may be increased in some benign conditions (8, 9). Guidelines suggest that measurement of TM in serum is not an adequate screening tool, with the exception of CA125 which is recommended for the diagnosis of ovarian cancer in women at high risk for the disease. However, TM play an important role in the monitoring of patients undergoing treatment (10, 11).

In physiological conditions within the CNS, no cells are able to produce TM. Increased concentrations of CEA (12, 13), α-fetoprotein and β-human chorionic gonadotropin (βHCG) (14) have been observed in the CSF of patients with LM, but the amount of TM in CSF due to filtration and/or to dysfunction of the blood/CSF barrier was not always evaluated correctly (15). In most cases, TM levels have been considered diagnostic for LM when concentrations in CSF were greater than those observed in serum (16).

In the present work, we evaluated intrathecal synthesis of CEA, CA15.3, CA125 and CA19.9 in 18 carcinoma patients with LM diagnosed by positive CSF cytology, and in 50 patients with other neurological diseases (OND). Intrathecal synthesis was calculated according to the mathematical approach suggested by Reiber for immunoglobulins (17, 18).

Vascular endothelial growth factor (VEGF) is a proangiogenic factor and a vascular permeability factor, playing a critical role in tumor angiogenesis (19). An increase in VEGF plasma concentrations was described in patients with different solid tumors. It has been reported that VEGF could be a useful biological marker for detection of carcinomatous meningitis (20, 21).

In the same 18 LM patients and in a subgroup of 26 OND patients, VEGF concentrations were measured in serum and CSF. We performed these measurements in order to evaluate if the VEGF Index may provide additional information about diagnosis and/or prognosis in suspected LM.

Materials and methods

Patients

Eighteen patients with positive CSF cytology for LM were investigated. The demographics of the patients are reported in Table 1. A systemic tumor had already been diagnosed in 15 patients, nine of which were diagnosed more than 3 years before LM. In three patients (patients 4, 12 and 16), neurological symptoms due to LM were the first clinical manifestations of cancer. In patient 13, a definitive diagnosis of LM was possible following a second lumbar puncture.

To evaluate the specificity of intrathecal synthesis of TM, 50 patients with non-malignant neurological diseases (OND) were included in the study as the control group (24M, 26F, mean age 51, range 19–74). OND patients were affected by inflammatory (n = 33) or neurodegenerative diseases (n = 17).

CSF and blood samples were collected by lumbar puncture and by venous puncture, respectively.

Examination of CSF included cell count, glucose, total protein and albumin quotient (QAlb = CSF albumin/serum albumin × 1000). The latter parameter is recommended to evaluate function of the blood/CSF barrier. All CSF parameters were analyzed within 2 h of collection. Cytospin for cytological analysis was also performed within 2 h.

For quantitation of TM and VEGF, sera and cell-free CSF were aliquoted, stored at –80°C and analyzed within 3 months.

Cytological examination was performed for both LM and OND patients.

Biochemical analysis

Serum and CSF CEA, CA15.3, CA125 and CA19.9 concentrations were evaluated using Modular Analytics SWA (Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection for CEA, CA15.3, CA125 and CA19.9 was 0.2 ng/mL, 1 UI/mL, 0.6 UI/mL, and 0.6 UI/mL, respectively. The lower limit of detection was validated using 20 CSF samples from OND patients with normal biochemical parameters.

VEGF concentrations were measured in both serum and CSF in all patients with LM, and in a subgroup of 26 patients (18 patients showing CFS/blood barrier dysfunction, i.e., QAlb > 8, 10 F, 8 M, 12 inflammatory diseases and 6 neurodegenerative; 8 patients with QAlb < 8, 5 F, 3 M, 6 inflammatory diseases and 2 neurodegenerative). VEGF concentrations were measured using a commercially available ELISA assay with a detection limit of < 5 pg/mL (R&D Systems, Minneapolis, MN, USA).

Intrathecal TM synthesis

Intrathecal synthesis was calculated using a mathematical approach that identifies the amount of tumor markers detected in CSF due to filtration from blood and/or barrier dysfunction (17). For intrathecal TM synthesis, was applied the Reiber's formula for evaluation of intrathecal IgA synthesis, since the molecular weight of the analytes (i.e., CEA: 200 kDa, CA125: 500 kDa, CA15.3: 300–450 kDa, CA19.9: 360 kDa) (22) is similar to that of IgA (18).

TM Intrathecal synthesis (TM_{IS}) was assessed as follows:

$$TM_{IS} = [Q_{Tm} - Q_{Lim}\ (TM)] \times TM_{serum}/1000$$

$$(Q_{Tm} = TM_{CSF}/TM_{Serum} \times 1000)$$

$$Q_{Lim}(TM) = a/b\sqrt{QA1b^2 + b^2} - c$$

Table 1 Demographics of LM patients.

Patients	Primary tumor	Time from diagnosis, years	Cerebral metastasis	Age	Gender
Patient 1	Small-cell lung cancer	0–3	–	55	M
Patient 2	Breast carcinoma	> 3	–	55	F
Patient 3	Small-cell lung cancer	0–3	–	50	F
Patient 4	Small-cell lung cancer	–	Yes	46	M
Patient 5	Cervix carcinoma	0–3	–	42	F
Patient 6	Breast carcinoma	> 3	–	45	F
Patient 7	Small-cell lung cancer	0–3	–	59	F
Patient 8	Breast carcinoma	> 3	–	63	F
Patient 9	Breast carcinoma	> 3	–	46	F
Patient 10	Breast carcinoma	> 3	–	51	F
Patient 11	Breast carcinoma	–	–	52	F
Patient 12	Breast carcinoma	–	–	67	F
Patient 13	Breast carcinoma	> 3	–	56	F
Patient 14	Colon carcinoma	0–3	Yes	67	M
Patient 15	Breast carcinoma	> 3	Yes	36	F
Patient 16	Gastric carcinoma	> 3	–	61	F
Patient 17	Breast carcinoma	> 3	–	68	F
Patient 18	Breast carcinoma	0–3	–	55	F

Data refer to time of diagnosis of LM. In patient 4, patient 11 and patient 12, LM was the first manifestation of systemic cancer.

$(Q_{Lim}(TM) = 0.77 \sqrt{QAlb^2 + 23 \times 10^{-6} - 3.1 \times 10^{-3}})$

Q_{Lim} is the upper discrimination limit of the reference range, indicating no synthesis in the Reiber graphs.

The parameters $a/b = 0.77$, $b^2 = 23 \times 10^{-6}$ and $c = 3.1 \times 10^{-3}$ are referred to IgA and depend only on the molecular weight.

When the TM concentration in CSF due to intrathecal production was >10% as compared to Q_{Lim} (cut-off >10%), samples were considered positive for intrathecal synthesis.

The VEGF Index was calculated as follows:

$$\text{VEGF Index} = (VEGF_{CSF}/VEGF_{serum})/(ALB_{CSF}/ALB_{serum})$$

Statistical analysis

Statistical analysis of VEGF Index was performed using the Mann-Whitney test and χ^2 test. The level of significance was set at 0.05.

Results

All LM patients showed blood/CSF barrier dysfunction (QAlb>8). Nine of 18 patients (50%) showed decreased glucose concentrations in CSF (reference range: 40–80 mg/dL), 17 of 18 (94%) displayed mild pleiocytosis (Table 2). Intrathecal synthesis for at least one TM was detected in all LM patients that were evaluated (Table 3A), while none of the OND patients displayed TM production in CSF.

Intrathecal synthesis of CEA was observed in 17 of 18 patients (percentage of CEA of intrathecal production, range: 92%–100%), CA125 in nine of 18 (range: 38%–100%), CA15.3 in 15 of 18 (range: 53%–100%) and CA19.9 in 12 of 18 (range: 51%–100%). In addition, 16 of 18 patients were positive for two or more TM, 13 of 18 were positive for three or more markers and six of 18 were positive for all TM.

Table 2 CSF values for glucose (reference range: 40–80 mg/dL), QAlb (CSF/serum albumin ratio), cell count and tumor cells, evaluated in cytological analysis, in LM patients.

Patients	CSF glucose, mg/dL	QAlb	Total cells/μL	Tumor cells/μL
Patient 1	85	20.32	8	3
Patient 2	41	25.4	8.3	0.6
Patient 3	<2	21.1	31	22
Patient 4	44	13.4	23	1
Patient 5	<2	19.6	85	25
Patient 6	67	215.8	13	2
Patient 7	32	43.9	34	10
Patient 8	29	21.7	94	3
Patient 9	11	28.5	35	6
Patient 10	51	135.9	8.6	1
Patient 11	39	25.2	13	5
Patient 12	53	76.9	3	0.6
Patient 13	32	34.11	12	0
Patient 14	40	16.4	4.3	0.3
Patient 15	53	63.1	12	0.6
Patient 16	11	10.8	40	20
Patient 17	30	27.3	25	1
Patient 18	41	9.82	34	30

Data for patient 13 refer to the first lumbar puncture which was negative for tumor cells. Conversion factor glucose (mg/dL) × 0.0555 = glucose (mmol/L).

CSF TM concentrations were measurable in two OND patients (OND patient 1: CSF CA19.9: 1.62 UI/mL, serum CA19.9: 33.2 UI/mL, QAlb: 129; and OND patient 2: CSF CA125: 1.16 UI/mL, serum CA125: 91.7 UI/mL, QAlb 21). However, no intrathecal synthesis was detectable (OND patient 1: $CA19.9_{IS} = -0.76$, and OND patient 2: $CA125_{IS} = -0.84$, $< Q_{lim}$). This suggests that the increase in TM CSF concentration observed in these patients was due to both high serum concentrations and/or to dysfunction of the CSF/blood barrier.

No correlation was found between TM and tumor type. As a matter of fact, patient 13, diagnosed with breast carcinoma, did not show intrathecal synthesis of CA15.3, which is more frequently associated with breast cancer. Also, patient 5, diagnosed with uterine carcinoma, showed CSF CA19.9 values higher than CA125, which is closely related to ovarian carcinoma.

Patient 13, with a negative cytological examination at the initial lumbar puncture had intrathecal synthesis for CEA, CA125 and CA19.9. A second lumbar puncture performed on this patient revealed positive cytology, confirming the diagnosis of LM.

Only four patients were monitored for intrathecal synthesis of TM at different time points. These patients were monitored during intrathecal treatment with methotrexate and/or liposomal cytosine-arabinoside ARA-C (Depocyte®). Three patients showed only slight fluctuations in TM (data not shown). However, patient 10, following 6 months of therapy (first with intrathecal methotrexate, 15 mg, and then with Depocyte, 50 mg), showed negative CSF cytology which paralleled the absence of intrathecal synthesis of CEA and CA15.3. After more than 2 years, the patient remains clinically stable. Serum concentrations of CEA, CA15.3, CA125 and CA19.9, are reported in Table 3B.

With respect to the VEGF Index, no significant differences were observed between the two groups of OND patients (i.e., OND with QAlb>8, mean: 4.7, range 0–24.4; OND QAlb<8, mean: 4.5, range 1.1–16). Thus, OND patients were then analyzed as a single group which showed a mean of 4.6 (range 0–24.4).

The VEGF Index was significantly increased in LM patients as compared to OND patients (mean LM: 117.3, range 4.05–628; $p < 0.001$) (Table 3A).

Setting the VEGF Index cut-off value at 10 revealed a sensitivity of 83.3% and specificity of 88.4%. A statistically significant difference was detected between LM and OND patients ($p < 0.025$, χ^2 test).

The significant overlap between LM and OND patient values underlines the lower diagnostic specificity of this test when compared to cytological examination or intrathecal TM synthesis.

Discussion

The search for additional laboratory tests improving the diagnosis of LM is an important issue for CSF

Table 3A TM intrathecal synthesis and VEGF Index in LM patients.

Patients	CEA		CA125		CA15.3		CA19.9		VEGF Index
	ng/mL	%	IU/mL	%	IU/mL	%	IU/mL	%	
Patient 1	7.83	98	n.d.	0	6.05	74	2.63	91	7.81
Patient 2	0.3	68	0.6	38	2.02	87	0.58	83	120.92
Patient 3	0.93	89	n.d.	0	2.53	88	0.62	68	207
Patient 4	11.27	100	n.d.	0	n.d.	0	2786.1	100	4.05
Patient 5	12.63	99	2513.3	99	17.32	98	10203.8	100	396
Patient 6	115.0	96	34.4	96	209.6	70	0	0	11.42
Patient 7	181.9	99	26.5	98	0.9	75	14.75	98	28.85
Patient 8	1.87	99	n.d.	0	57.7	97	0.87	51	81.09
Patient 9	6.31	99	1.38	79	14.71	95	1135.5	100	141.58
Patient 10	0.75	86	n.d.	0	1.3	45	n.d.	0	6.48
Patient 11	n.d.	0	n.d.	0	30.46	89	n.d.	0	22.14
Patient 12	1.85	93	n.d.	0	72.29	96	0	0	43.87
Patient 13	34.14	100	2.43	71	n.d.	0	1.02	84	13.29
Patient 14	536	100	n.d.	0	n.d.	0	n.d.	0	628.04
Patient 15	31.6	92	13.3	89	1.33	53	5.4	79	18.52
Patient 16	48.75	100	1217.5	100	5.47	98	999	100	99.4
Patient 17	253.9	100	n.d.	0	4.7	94	244.4	100	184.8
Patient 18	2.04	100	12.6	99	27.8	100	n.d.	0	95.41

TM_{IS}, calculated according to Reiber's formula, is reported as both concentration and percentage (cut-off: >10%). CEA was positive in 17/18, CA125 in 9/18, CA15.3 in 15/18, CA19.9 in 12/18 patients. The VEGF Index was significantly increased in LM patients as compared to OND patients (mean LM: 117.3 vs. mean OND: 4.6, $p<0.001$). n.d., not detectable; 0, no intrathecal synthesis but detectable CSF amounts, due to high QAlb or high TM concentration in serum.

analysis. A number of different biological markers such as lactate dehydrogenase (LDH) and β-glucuronidase have been proposed (2). However, the lack of sensitivity and specificity of these analytes limits their use in diagnostic practice.

Applying the mathematical approach suggested by Reiber (17, 18) we evaluated the sensitivity and the specificity of intrathecal synthesis of CEA, CA125, CA15.3 and CA19.9 in 18 patients with diagnosed LM from solid cancers, and in a control group. We compared sensitivity and specificity of intrathecal synthesis of TM for LM detection with those of VEGF Index.

Our data show that intrathecal synthesis of TM is 100% specific for LM. In fact, no OND patients displayed TM synthesis in the CSF compartment, whereas all LM patients were positive. In addition, TM_{IS} proved to be as sensitive as cytological examination. Also, we measured TM using with an automated analyzer (i.e., Modular Analytics SWA, Roche), but better results could be obtained using methods with a lower detection limit, such as ELISA.

Table 3B Serum concentration of TM in LM patients.

Patients	CEA, ng/mL	CA125, IU/mL	CA15.3, IU/mL	CA19.9, IU/mL
Patient 1	14.9	54.04	160.8	20.16
Patient 2	7.24	56.74	18.1	7.28
Patient 3	8.01	10.09	25.14	22.46
Patient 4	3.39	10.55	22.73	364.4
Patient 5	4.86	1256	22.57	2572
Patient 6	42.61	42.55	393	19.87
Patient 7	35.7	17.69	9.69	9.46
Patient 8	2.78	7.29	124.1	17.71
Patient 9	4.2	18.9	41.18	234.8
Patient 10	1.2	8.6	16.3	6.3
Patient 11	2.13	15.4	246.8	14.24
Patient 12	2.66	20.5	58.85	57.69
Patient 13	6.47	41.4	8.29	8.24
Patient 14	123.5	12.3	19.87	9.24
Patient 15	41.3	17.4	36.4	25.7
Patient 16	7.62	86.2	20.42	169.5
Patient 17	3.7	12.2	18	29.65
Patient 18	2.48	26	32	10

Reference range: CEA, 0–10 ng/mL; CA125, 0–35 IU/mL; CA15.3, 0–31.3 IU/mL; CA19.9, 0–37 IU/mL.

We found intrathecal release of TM in all LM patients studied. There was no clear correlation with the type of carcinoma. Since CEA was detected in almost all patients (17/18), this should be considered the marker of choice as a diagnostic test for LM. Quantitation of CA125, CA15.3 and CA19.9 may be performed only in those cases with negative intrathecal synthesis of CEA.

According to some authors, CSF analysis of TM such as CEA, βHCG or α-fetoprotein can occasionally be useful in diagnosis of LM when CSF concentrations are above a pre-determined cut-off value (15), or when CSF values are higher than serum values (16), in the absence of dysfunction of the blood/CSF barrier, or when TM are disproportionately increased in the CSF (23).

The concentration of a specific protein in CSF is dependent on its concentration in blood and on CSF/blood barrier integrity. Protein entry from blood into the CSF follows the laws of diffusion as a function of molecular size. Thus, the choice of a pre-determined cut-off value in CSF is inadequate (i.e., CEA < 4 μg/L; 15). In addition, since a higher concentration in CSF compared to serum in at least one TM was present in 11 out of 18 patients with LM, use of the above-mentioned approaches to define abnormal CSF levels of the markers would significantly decrease the test sensitivity. Since alterations in blood/CSF barrier function are found in ~80% of LM patients (2), the correct approach should consider all these variables. Our

data further suggest that the well-known hyperbolic function of Reiber graphs (17, 18) could be applied to TM, and intrathecal synthesis of TM may be an effective diagnostic tool.

In addition to laboratory tests, neuroradiological techniques, especially gadolinium-enhanced magnetic resonance imaging (MRI) which is more sensitive than computed tomography (CT), can be important diagnostic tools.

Although neuroradiological findings may be associated with other non-neoplastic diseases, they are often highly suggestive for LM diagnosis in an appropriate clinical context.

Concerning the relationship between TM concentrations and disease course, correlation between TM concentrations and disease progression was previously reported by Kosmas and co-workers (24, 25). These investigators monitored CEA, CA15.3, CA125 and CA19.9 concentrations in serum and CSF of five breast cancer patients with carcinomatous meningitis.

In our study, only four LM patients were monitored over time. Interestingly, patient 10, who showed mild intrathecal synthesis of two markers when diagnosed for LM, displayed negative cytology and no intrathecal synthesis of TM after 6 months of intrathecal treatment with ARA-C. This patient remains clinically stable 2 years after diagnosis of LM. However, this single finding does not allow any conclusion to be made about the potential prognostic value of TM.

Also, the VEGF Index was significantly higher in LM patients compared with controls. However, production of VEGF in the CSF compartment was observed in most of the OND patients, although at lower levels. It should be noted that VEGF may increase in infectious meningitis (26, 27) and in other inflammatory conditions (28). In our study, the VEGF Index per se did not increase diagnostic information, since both its specificity and sensitivity were lower than those of TMs. Further studies are needed to investigate the possible use of the VEGF Index for the detection of LM dissemination of solid tumors such as melanoma or glioma.

Other authors have previously suggested a mathematical model for discriminating LM from non-LM patients measuring VEGF, tissue plasminogen activator (tPA) and transforming growth factor-β (TGF-β) concentrations in CSF and serum (28). However, this intriguing approach seems too difficult to apply in the diagnostic routine.

The paucity and fragility of tumor cells in the CSF is the main cause of low-sensitivity of cytological examination. It is well known that delayed processing of samples may increase the incidence of false-negative cytology (29). CSF for cytological examination should be processed within 2 h, while TM are stable for at least 5 days at 2°C–8°C, and for months if stored at –20°C according to the manufacturer's instruction and our own experience. Quantitation of glycoproteins such as CEA, CA15.3, CA125 and CA19.9 that are more stable than cells can be particularly helpful. In addition, quantitation is possible even in mishandled samples (i.e., delay in CSF processing).

The experience of the cytopathologist is another important aspect for the correct diagnosis of LM. For this reason, only some hospitals can afford this kind of analysis. On the contrary, TM measurements require equipment that is often available in most laboratories.

Our observations, obtained on 18 patients with LM should be considered preliminary. Further studies, currently in progress with a larger cohort of patients, are needed to confirm these findings and to assess their prognostic values. However, intrathecal synthesis of TM, calculated according to Reiber formula, is a specific and sensitive parameter. This parameter can be obtained using reproducible and standardized methods, and can be used in laboratories for clinical investigation.

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