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Optimal cut-off values for tumor markers in cerebrospinal fluid with ROC curve analysis

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1. ABSTRACT

To select optimal cut-off values of tumor markers in cerebrospinal fluid for the diagnosis of meningeal carcinomatosis, the concentrations of CEA, CA125, CA153, CA199, CA724, CYFRA21-1, AFP and NSE were determined by means of double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) in 21 MC patients, 25 cancer patients without leptomeningeal disease (group one) and 45 meningitis patients (group two) using the Roche E170 modular immunoassay analyzer. Optimal cut-off values were selected based on a receiver-operating characteristic curve. The results showed that CA125 and CEA in CSF were optimal diagnostic indices distinguishing between MC patients and cancer patients without leptomeningeal disease. Cut-off values for CA125 and CEA were 1.715 μ/ml and 0.274 μg/l, respectively. In addition, CEA in CSF was the optimal diagnostic index distinguishing MC patients from meningitis patients. The cut-off value for CEA was 4.522 μg/l.

2. INTRODUCTION

Tumor markers in cerebrospinal fluid may be used for the early diagnosis of meningeal carcinomatosis (1-2). However, normal reference values for these markers have not yet been established. In clinical practice, concentrations of tumor markers in serum are often used as references. To test the optimal cut-off values for tumor markers in CSF, statistical analyses were conducted in 21 MC patients, 25 cancer patients without leptomeningeal disease and 45 meningitis patients. For all patients levels of tumor markers in serum and CSF were measured in our hospital between January 2004 and February 2010.

3. MATERIALS AND METHODS

3.1. Clinical data

3.1.1. Introduction of 21 MC patients

Twenty-one MC patients were clearly diagnosed based on CSF cytology or typical brain magnetic resonance

Table 1. Age composition of three groups

Group	N	≤50	>50	$\overline{X} \pm s$	Age range
MC	21	10(5:5)	11(3:8)	50.143±9.345	23-65
Comparison group one	25	7(4:3)	18(13:5)	55.120±10.325*	37-83
Comparison group two	45	25(11:14)	20(9:11)	46.311±15.807*	19-75

* represents p>0.05, (number: number) represents (male: female)

Table 2. Comparison of tumor markers in CSF among three groups.(* represents MC compared with group one, p<0.05 Δ represents comparison group one compared with group two, p<0.05)

Items	MC	Comparison group one	Comparison group two
CEA(μg/l)	76.770+199.690*	0.200+0.000	0.200+0.065
CA125(μ/ml)	2.650+200.155*	0.600+0.460 $^{\Delta}$	1.406±0.650
CA153(μ/ml)	2.660+4.050 *	1.000+0.000	1.000+0.170
CA199(μ/ml)	1.890+5.290	1.401+1.470$^{\Delta}$	0.600+0.785
CA724(μ/ml)	0.851+0.896*	1.140+0.220$^{\Delta}$	0.944±0.366
CYFRA21-1(ng/ml)	3.720+9.623*	1.150+0.560$^{\Delta}$	0.827+0.705
AFP(μg/l)	0.605+0.143	0.605+0.000	0.605+0.000
NSE (ng/ml)	14.750+13.180*	11.380+8.665	8.855+7.950

images (MRIs). Primarily tumors include pulmonary carcinoma (16/21, 76.0%), gastric cancer (2/21, 9.6%), thyroid carcinoma (1/21, 4.8%), lymphadenoma (1/21, 4.8%) and breast carcinoma (1/21, 4.8%). All samples including serum and CSF were analyzed during the early course of the disease (one to six days). No patients were treated with intrathecal chemotherapy.

3.1.2. Introduction of 25 cancer patients without leptomeningeal disease

Comparison group one was comprised of 25 cancer patients without leptomeningeal disease which included pulmonary carcinoma (14/25, 56.0%), digestive system cancer (6/25, 24.0%), hematological system cancer (2/25, 8.0%), endocrine system cancer (2/25, 8.0%) and nasopharyngeal carcinoma (1/25, 4.0%). All patients were clearly diagnosed based on pathology.

3.1.3. Introduction of 45 meningitis patients

Comparison group two was comprised of 45 meningitis patients. There were 12 cases of viral meningitis, 20 cases of tuberculous meningitis, 3 cases of purulent meningitis, 1 case of cryptococcal meningitis and 9 cases of indefinite pathogenic bacteria meningitis. All patients were cured through clinical treatment.

3.2. Sampling

Signed consent informs were obtained from all patients. Through lumbar puncture, 3 ml of fresh CSF and 4 ml of serum were collected for determination of tumor marker levels.

3.3. Concentration determinations

Concentrations of CEA, CA125, CA153, CA199, CA724, CYFRA21-1, AFP and NSE were determined by means of double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) using the Roche E170 modular immunoassay analyzer.

3.4. Statistical analyses

Normally distributed data were expressed b $\overline{X} \pm s$. Data with a skewed by median and quartile. The Kruskal-Wallis test was used to compare data consistency and tumor markers in serum and CSF across the three groups. Optimal cut-off values for tumor markers in CSF were determined based on Receiver-operating characteristic (ROC) curve analysis. For all tests, a significance level of <0.05 was used. SPSS for Windows 13.0 was used for all statistical analyses.

4. RESULTS

4.1. Data consistency check among three groups

Results shown in Table 1: Age was not significantly different between MC patients and the other two comparison groups (average rank difference =9.60, 5.09, p>0.05).

4.2. Comparison of tumor markers in CSF and serum among three groups

4.2.1. Comparison of tumor markers in CSF among three groups

Results shown in Table 2: CEA, CA125, CA153, CA724, CYFRA21-1 and NSE levels were significantly different between MC patients and comparison group one (p<0.05). The significant differences in the levels of all markers except CA724 were measured between MC patients and comparison group two (p<0.05). CA125, CA199, CA724 and CYFRA21-1 levels were significantly different between comparison one and comparison group two (p<0.05).

4.2.2. Comparison of tumor markers in serum among three groups

Results shown in Table 3: CEA, CA125, CA153 and CA199 levels were significantly different between MC patients and comparison group one (p<0.05). CEA, CA125, CA153, CA199 and AFP levels were significantly different between MC patients and comparison group two (p<0.05). Only CYFRA21-1 was present at significantly different levels between comparison group one and comparison group two (p<0.05).

4.3. MC patients compared with group one through ROC curve

4.3.1. Area under the curve with ROC curve analysis

Results shown in Table 4 and Figure 1: CEA, CA125, CA153, CA724, CYFRA21-1 and NSE had diagnostic value between these two groups (p<0.05), while

Table 3. Comparison of tumor markers in serum among three groups.(* represents MC compared with group one, $p<0.05$, $^{\Delta}$ represents comparison group one compared with group two, $p<0.05$)

Items	MC	Comparison group one	Comparison group two
CEA(μg/l)	54.260+251.550*	2.115+1.123	1.840+0.815
CA125(μ/ml)	38.555+75.555*	12.540+8.565	11.890+9.100
CA153(μ/ml)	15.920+19.260*	8.500+7.218	9.160+7.055
CA199(μ/ml)	25.415+15.442*	10.365+7.720	7.995+6.740
CA724(μ/ml)	2.210+3.389	1.600+1.245	1.380+2.554
CYFRA21-1(ng/ml)	1.960+4.140	2.339±1.034$^{\Delta}$	1.475+0.877
AFP(μg/l)	3.210+2.335	2.695+1.925	2.290+1.780
NSE (ng/ml)	13.176±4.413	11.280+6.585	8.660+6.060

Table 4. Area under the curve with ROC curve analysis

Items	Area	Standard Error	95% Confidence Interval	P value
CEA(μg/l)	0.930	0.043	(0.845 1.014)	0.000
CA125(μ/ml)	0.971	0.024	(0.925 1.018)	0.000
CA153(μ/ml)	0.850	0.077	(0.699 1.001)	0.001
CA199(μ/ml)	0.613	0.107	(0.403 0.824)	0.277
CA724(μ/ml)	0.293	0.121	(0.055 0.531)	0.045
CYFRA21-1(ng/ml)	0.750	0.107	(0.541 0.959)	0.014
AFP(μg/l)	0.602	0.103	(0.399 0.805)	0.315
NSE (ng/ml)	0.717	0.092	(0.537 0.896)	0.040

Table 5. Optimal cut-off values for tumor markers in CSF

Items	cut-off values	Sensitivity	Specificity	Accuracy	Youden's index
CEA(μg /l)	0.274	0.905	0.880	0.891	0.785
CA125 (μ/ml)	1.715	0.933	0.952	0.944	0.886
CA153(μml)	1.005	0.769	0.900	0.848	0.669
CA724(μ/ml)	3.340	0.231	1.000	0.706	0.231
CYFRA21-1(ng/ml)	2.030	0.714	0.900	0.824	0.614
NSE(ng/ml)	12.300	0.769	0.632	0.688	0.401

AFP and CA199 were of no diagnostic value between these two groups ($p>0.05$).

4.3.2. Optimal cut-off values for tumor markers in CSF

Results shown in Table 5: Clinical value was determined for CA125, CEA, CA153, CYFRA21-1, NSE and CA724 according to Youden's index. Levels of CA125 and CEA were optimal diagnostic indices for which Youden's index was greater than 0.70.

4.4. MC patients compared with group two through ROC curve

4.4.1. Area under the curve with ROC curve analysis

Results shown in Table 6 and Figure 2: CEA, CA125, CA153, CA199, CYFRA21-1 and NSE had diagnostic value between these two groups ($p<0.05$), while AFP and CA724 were of no diagnostic value between these two groups ($p>0.05$).

4.4.2. Optimal cut-off values for tumor markers in CSF

Results shown in Table 7: Clinical value was determined for CEA, CYFRA21-1, CA125, CA153, CA199 and NSE according to Youden's index. CEA was the optimal diagnostic index, with a valve of greater than 0.70 for Youden's index.

5. DISCUSSION

ROC curve analysis is said to have originally developed during World War II to analyze classification accuracy in differentiating signal from noise in radar detection. The principles of ROC analysis were later implemented for improving medical decision making (3). Evidently, this methodology has been adapted to several medical areas that are dependent on the accuracy of screening and diagnostic tests, such as laboratory testing (4-5), epidemiology (6-7), radiology (8), several clinical disciplines (9-12), and bioinformatics (13-14).

Tumor markers consist of a cluster of substances that are synthesized and released by tumor cells during the development and reproduction of tumors or released due to host reactions to carcinomas. The presence of certain tumor marker may indicate the nature of tumor. From a clinical perspective, tumor markers may have a variety of functions that correspond to different stages in disease development. Analysis of tumor markers can assist in the care of patients who are asymptomatic, those who are suspected to have the disease and those with overt disease for whom therapy may or may not have been initiated. Analysis of tumor markers can also be used for determining treatment response or surveillance after therapy. The accuracy of analyzing tumor marker is the presupposition for its appropriate use for these purposes. Analyzing tumor markers must give clinicians the ability to correctly classify one condition and/or outcome from another (15). Because all the samples were obtained during early onset of MC (one to six days) and because patients were not treated with intrathecal chemotherapy, so statistical results could be used for early diagnosis.

Based on their chemical characteristics, tumor markers may be classified into embryonic antigen tumor markers, carbohydrate antigen tumor markers, enzyme

Table 6. Area under the curve with ROC curve analysis

Items	Area	Standard Error	95%Confidence Interval	P value
CEA(μg/l)	0.924	0.046	(0.833 1.015)	0.000
CA125(μ/ml)	0.872	0.050	(0.774 0.970)	0.000
CA153(μ/ml)	0.802	0.080	(0.645 0.959)	0.001
CA199(μ/ml)	0.739	0.096	(0.551 0.927)	0.010
CA724(μ/ml)	0.448	0.109	(0.234 0.662)	0.572
CYFRA21-1(ng/ml)	0.830	0.081	(0.670 0.990)	0.000
AFP(μg/l)	0.630	0.093	(0.447 0.813)	0.157
NSE (ng/ml)	0.707	0.075	(0.561 0.853)	0.025

Table 7. Optimal cut-off values for tumor markers in CSF

Items	cut-off values	Sensitivity	Specificity	Accuracy	Youden's index
CEA(μg /l)	4.522	0.810	1.000	0.939	0.810
CA125(μ/ml)	1.720	0.933	0.659	0.729	0.592
CA153(μ/ml)	1.695	0.615	0.927	0.852	0.542
CA199(μ/ml)	1.470	0.692	0.786	0.764	0.478
CYFRA21-1(ng/ml)	1.510	0.786	0.905	0.875	0.690
NSE (ng/ml)	9.155	0.923	0.524	0.618	0.447

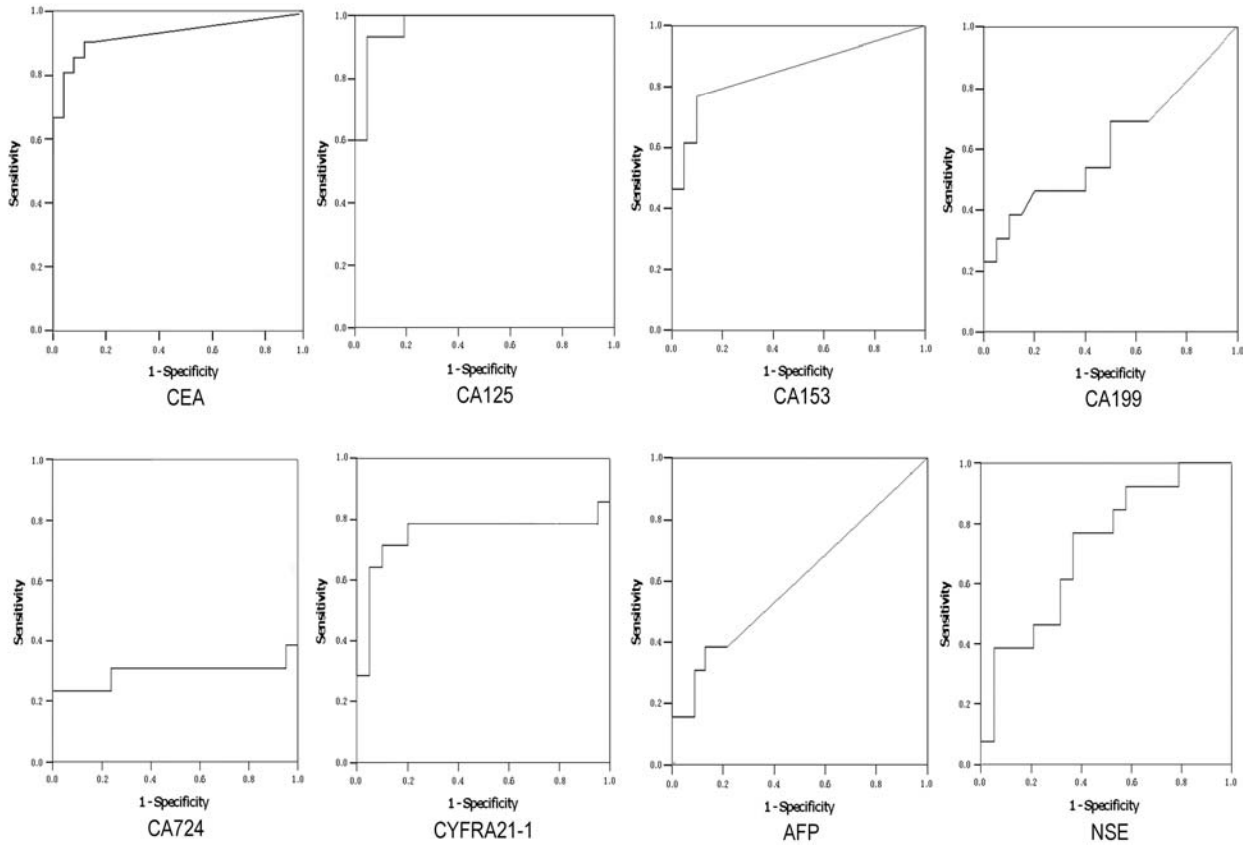

Figure 1. Compared 21 MC patients with 25 cancer patients without leptomeningeal disease. CEA, CA125, CA153, CA724, CYFRA21-1 and NSE had diagnostic value, while AFP and CA199 were of no diagnostic value. Statistical analysis was performed by ROC curve.

tumor markers, hormone tumor markers, protein tumor markers and gene tumor markers. CEA and AFP are embryonic antigen tumor markers CA125, CA153, CA199, CA724 and CYFRA21-1 are carbohydrate antigen tumor markers, while NSE is an enzyme marker (16). Different biomarkers are sensitive to different diseases. AFP is sensitive to primary hepatic carcinoma while CA724 is sensitive to gastric cancer. Primarily tumors in MC patients are different. Because the origin of this group is almost certain, AFP levels in CSF or serum are of no diagnostic value for distinguishing between MC patients and cancer patients without leptomeningeal disease.

There are two possible mechanisms to explain the elevation of tumor markers in CSF. One possibility is that tumor markers are locally produced by cancer cells.

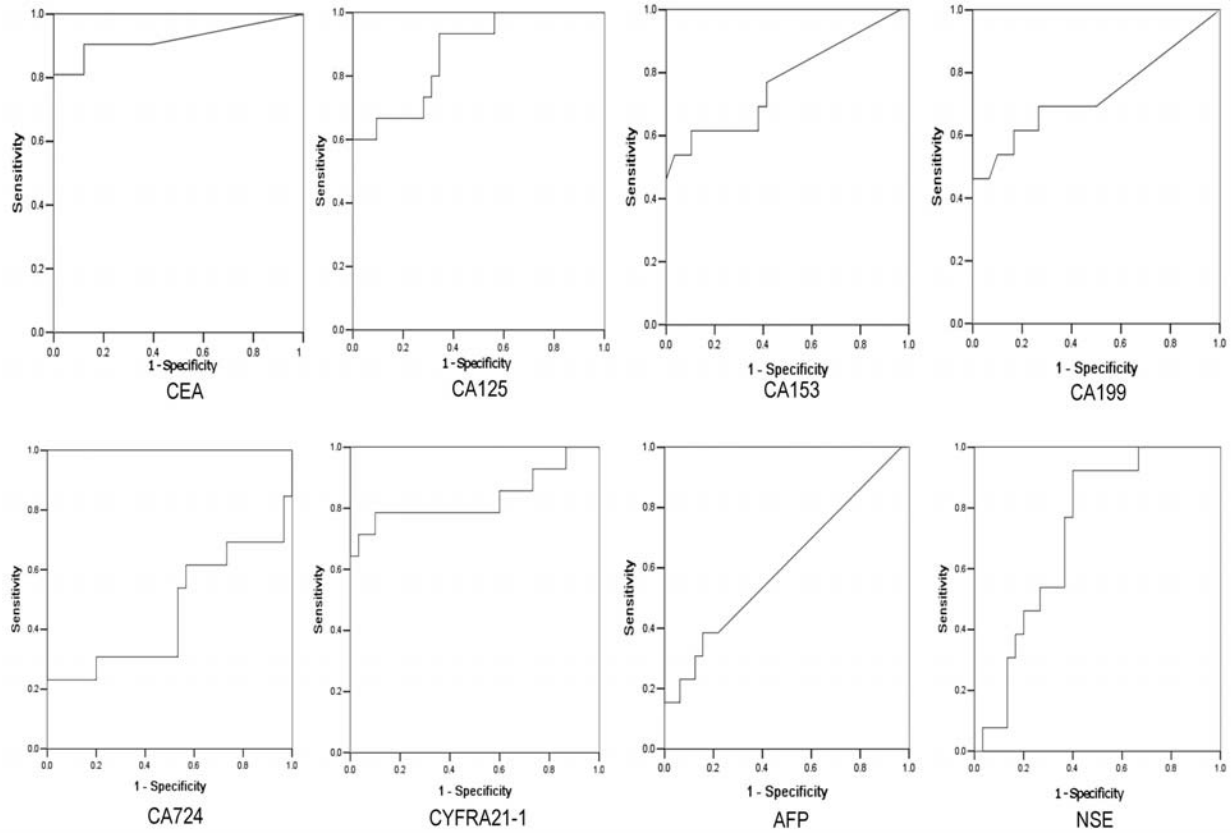

Figure 2. Compared 21 MC patients with 45 meningitis patients. CEA, CA125, CA153, CA199, CYFRA21-1 and NSE had diagnostic value, while AFP and CA724 were of no diagnostic value. Statistical analysis was performed by ROC curve.

Alternatively, Tumor markers could be passively transferred to serum, reflecting elevated serum tumor markers levels and/or a blood-CSF-barrier disturbance. This may cause differences between CSF and serum levels when MC comparing MC patients to cancer patients without leptomeningeal disease.

6. REFERENCES

1. Yasumoto K, Takahashi Y, Mai M, etal. Meningeal carcinomatosis preceded by a rapid increase in serum CA19-9 levels in a patient with breast cancer. *Int J Clin Oncol* 10, 276-280 (2005)

2. Miura M, Iijima N, Hayashida K, et al. Case of leptomeningeal carcinomatosis effectively treated with intrathecal chemotherapy using ventriculoperitoneal shunt. *Rinsho Shinkeigaku* 46, 404-409 (2006)

3. Lusted LB. Decision-making studies in patient management. *N Engl J Med* 284, 416-424 (1971)

4. Obuchowski NA, Lieber ML, Wians FH Jr. ROC curves in clinical chemistry: uses, misuses, and possible solutions. *Clin Chem* 50, 1118-1125 (2004)

5. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 39, 561-577 (1993)

6. Pepe MS, Janes HE. Gauging the performance of SNPs, biomarkers, and clinical factors for predicting risk of breast cancer. *J Natl Cancer Inst* 100, 978-979 (2008)

7. Walter SD, Sinuff T. Studies reporting ROC curves of diagnostic and prediction data can be incorporated into meta-analyses using corresponding odds ratios. *J Clin Epidemiol* 60, 530- 534 (2007)

8. Obuchowski NA. ROC analysis. *AJR Am J Roentgenol* 184, 364-372 (2005)

9. Zou KH, O'Malley AJ, Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation* 115, 654-657 (2007)

10. Musial J, Swadzba J, Motyl A, et al. Clinical significance of antiphospholipid protein antibodies. Receiver operating characteristics plot analysis. *J Rheumatol* 30, 723-730 (2003)

11. Cheung R, Altschuler MD, D'Amico AV,et al. Using the receiver operating characteristic curve to select

pretreatment and pathologic predictors for early and late postprostatectomy PSA failure. *Urology* 58, 400-405 (2001)

12. Körner H, Söreide JA, Söndenaa K. Diagnostic accuracy of inflammatory markers in patients operated on for suspected acute appendicitis: a receiver operating characteristic curve analysis. *Eur J Surg* 165, 679-685 (1999)

13. Li J, Fine JP. ROC analysis with multiple classes and multiple tests: methodology and its application in microarray studies. *Biostatistics* 9, 566-576 (2008)

14. Zheng Y,Cai T, Feng Z. Application of the time-dependent ROC curves for prognostic accuracy with multiple biomarkers. *Biometrics* 62, 279-287 (2006)

15. Søreide K. Receiver-operating characteristic curve analysis in diagnostic, prognostic and predictive biomarker research.*J Clin Pathol* 62, 1-5 (2009)

16. Shi Q, Pu CQ, Wu WP,etal. The determination of medical reference values for tumor markers in cerebrospinal fluid *Zhonghua Yi Xue Za Zhi*. 89, 355-356 (2009)

Abbreviations: ROC curve: receiver-operating characteristic curve, MC: meningeal carcinomatosis, CSF: cerebrospinal fluid

Key Words: Tumor markers, Receiver-operating characteristic curve, Cerebrospinal fluid

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