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RAPID COMMUNICATION

Value of carcinoembryonic antigen and cytokeratins for the detection of recurrent disease following curative resection of colorectal cancer

Luís C Fernandes, Su B Kim, Sarhan S Saad, Delcio Matos

Luís C Fernandes, Su B Kim, Sarhan S Saad, Delcio Matos, Coloproctology Sector, Department of Surgical Gastroenterology, Federal University of São Paulo-Escola Paulista de Medicina, São Paulo, Brazil
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Correspondence to: Luís César Fernandes, MD, UNIFESPEPM, Alameda Santos, n. 211, conj. 304, Paraíso, São Paulo, SP01419-000, Brazi. luiscfernandes@terra.com.br
Telephone: +55-11-32877231 Fax: +55-11-32511662
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Abstract

AIM: To evaluate the efficacy of postoperative serial assay of carcinoembryonic antigen (CEA) and cytokeratins for the detection of recurrent disease in patients with colorectal adenocarcinoma after radical surgery.

METHODS: Between 1993 and 2000, 120 patients with colorectal adenocarcinoma underwent radical surgery in the Department of Surgical Gastroenterology, Federal University of São Paulo-Escola Paulista de Medicina, São Paulo, Brazil. Periodic postoperative evaluation was performed by assaying markers in peripheral serum, colonoscopy and imaging examination. Presence of CEA was detected using the Delfia® method with 5 μg/L threshold, and cytokeratins using the LIA-mat® TPA-M Prolifigen® method with 72 U/L threshold.

RESULTS: In the first postoperative year, patients without recurrent disease had normal levels of CEA (1.5 ± 0.9 μg/L) and monoclonal tissue polypeptide antigen-M (TPA-M, 64.4 ± 47.8 U/L), while patients with recurrences had high levels of CEA (6.9 ± 9.8 μg/L, $P < 0.01$) and TPA-M (192.2 ± 328.8 U/L, $P < 0.05$). During the second postoperative year, patients without tumor recurrence had normal levels of CEA (2.0 ± 1.8 μg/L) and TPA-M (50.8 ± 38.4 U/L), while patients with recurrence had high levels of CEA (66.3 ± 130.8 μg/L, $P < 0.01$) and TPA-M (442.7 ± 652.8 U/L, $P < 0.05$). The mean follow-up time was 22.3 mo. There was recurrence in 23 cases. Five reoperations were performed without achieving radical excision. Rises in tumor marker levels preceded identification of recurrences: CEA in seven (30%) and TPA-M in eleven individuals (48%).

CONCLUSION: Intensive follow-up by serial assay of CEA and cytokeratins allows early detection of colorectal neoplasm recurrence.

Key words: Colorectal neoplasms; Cytokeratin; Carcinoembryonic antigen; Residual neoplasm

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INTRODUCTION

Serum tumor markers are substances that can be assayed in peripheral blood. Raised levels of these markers indicate the possible existence of neoplasia in the organism, and thus they form an important instrument in the therapeutic management of such patients[1]. In colorectal adenocarcinoma, carcinoembryonic antigen (CEA) discovered by Gold *et al*[2] in 1965, has been distinguished as a tumor marker with the best characteristics. It is an antigen with high serum levels at the embryonic stage and low levels in adult individuals, and forms the standard for different instances of evaluation[3,4]. Its diagnostic capacity oscillates around 40%[4]. It has a correlation with staging especially among patients with lesions at stage IV[4] of the TNM classification[5] and validity for determining prognostic indices[6]. It can be used as an instrument for determining tumor recurrence[7].

A variety of markers have been developed for better assessing patients with colorectal malignant neoplastic processes, such as CA 19-9[8], CA 242[9], CA 72-4[10], cytokeratins[11], vascular endothelial growth factor (VEGF)[12] and p53[13]. Of these, cytokeratins deserve special attention because of their characteristics. The first method developed for detecting cytokeratins was the tissue polypeptide antigen (TPA) method in 1978[11], evolving into the tissue polypeptide-specific antigen (TPS) method in 1992, using a reagent to identify the M3 epitope of the TPA molecule[14]. Subsequently, in 1994, monoclonal tissue polypeptide antigen (TPA-M) was developed with monoclonal antibodies directed against various epitopes of three different cytokeratins: 8, 18 and 19[15,16]. It has

been studied to evaluate whether it can be utilized for identifying patients with colorectal adenocarcinoma[15,16] and neoplasias in various other organs, such as prostate[17], ovary[18], lung[19], bladder[20] and breast[21].

The objective of the present study was to investigate the value of postoperative serial assay of cytokeratins and CEA, for detecting recurrences of tumors of the colon and rectum, with the aim of determining their possible clinical advantages.

MATERIALS AND METHODS

Patients

Between December 1993 and March 2000, 120 patients with colorectal adenocarcinoma underwent curative surgical treatment in the Department of Surgical Gastroenterology, Federal University of São Paulo-Escola Paulista de Medicina (UNIFESP-EPM), São Paulo, Brazil. Among the patients 69.2% were white, 20.1% brown, 7.7% yellow and 3% black, and 43.2% were male. At the time of diagnosis, their mean age was 62.16 years, ranging from 19 to 89 years. The location of the lesion was the rectum (54.4%), left colon (18.9%), transverse colon (3.6%) and right colon (23.1%). The mean diameter of the tumor was 6.1 cm, ranging from 1 to 17 cm.

The patients were treated according to a protocol approved by the Research Ethics Committee of the institution. The patients were made aware of the study, and signed a statement of consent to their participation in the investigation. Patients with other neoplasias on earlier occasions or those who received previous anti-neoplastic therapy were excluded from the investigation. The operation was considered curative or palliative in accordance with evaluation of the preoperative staging tests, intraoperative evaluation of whether there was residual macroscopic lesion, and verification of the anatomopathological report.

Methods

Staging of the neoplasias was done according to the TNM scheme. Stage I was found in 29, stage II in 21, stage III in 29, stage IV in 41 patients respectively[5]. It was proposed to the patients that evaluations should be made 6, 12, 18, 24, 36, 48 and 60 mo after the operation. Follow-up was performed by clinical assessment, laboratory tests and supplementary examinations such as colonoscopy, computerized tomography (CT) of the abdomen and pelvis, and radiography (X-ray) of the chest using posteroanterior (PA) and lateral views. Opaque enema, nuclear magnetic resonance (NMR) and bone scintigraphy were requested in accordance with the clinical indication in each case.

The CEA and TPA-M markers were evaluated for all patients in the study following the operation, and calculated by patient category (with or without recurrence of neoplasia) for each collection time. The initial collection of blood for assaying the tumor markers was performed while inducing anesthesia prior to the surgical operation. The blood samples were centrifuged with collection of peripheral serum, and stored at -20℃. The serum assays of tumor markers were performed in the Clinical Analysis Laboratory of Hospital São Paulo, UNIFESP-EPM. The CEA concentration was determined using the Delfia® method, taking 5 μg/L as the limit for normalcity[4,7]. Cytokeratins were quantified using the LIA-mat® TPA-M Prolifigen® method, taking 72 U/L as the limit for normality.

Statistical analysis

Statistical analysis was performed using receiver operator characteristic (ROC) curves[22], quantitative analysis of the variables and repeated-measurement variance analysis[23].

RESULTS

In the first postoperative year, patients without tumor recurrence had normal levels of CEA (1.5 ± 0.9 μg/L) and TPA-M (64.4 ± 47.8 U/L), while patients with recurrences had high levels of CEA (6.9 ± 9.8 μg/L, $P < 0.01$) and TPA-M (192.2 ± 328.8 U/L, $P < 0.05$) (Table 1). The serum levels of the tumor markers CEA and TPA-M were measured during the first postoperative year. These parameters could predict recurrences of neoplasia during the second postoperative year. The accuracy was 0.59 (0.43-0.76) for CEA and 0.76 (0.63-0.89) for TPA-M (Figure 1A).

The second postoperative year was the principal period for identifying tumor recurrence. During this year, the CEA levels were normal (2.0 ± 1.8 μg/L) in patients without tumor recurrence, and high (66.3 ± 130.8 μg/L, $P < 0.01$) in patients with recurrences (Table 1), with an accuracy of 0.68 (0.43 - 0.93) (Figure 1B). The circulating TPA-M levels remained normal (50.8 ± 38.4 U/L) in patients without tumor recurrence and high (442.7 ± 652.8 U/L, $P < 0.05$) in patients with recurrences (Table 1), with an accuracy of 0.78 (0.62-0.94) (Figure 1B).

Three (2.5%) out of the 120 patients were lost their follow-up after surgery. The mean time of follow-up was 22.3 mo, ranging 0.2-75 mo. Recurrence of neoplasia was observed in 23 patients (19.2%) during the study period, which was found by clinical, radiological and endoscopic examinations at an average of 18 mo after the initial operation, ranging from 6 to 48 mo. This was preceded by a rise in CEA in 7 patients (30%) and TPA-M in 11 patients (48%).

Among the 23 patients with recurrence of neoplasia, 18 (78.3%) presented multiple tumor recurrences, while 5 (21.7%) who were considered operable underwent surgery for the new lesions. They continued to present residual disease after surgery. Four of these patients died during the postoperative follow-up.

DISCUSSION

One year after the operation, the CEA level was around 1.5 μg/L in the patients without tumor recurrence and around 6.9 μg/L in those with tumor recurrence with an accuracy of 76%. The mean level of TPA-M was around 64.4 U/L in the patients without tumor recurrence and around 192.2 U/L in those with recurrence with an accuracy of 59.1%. In the second postoperative year, the mean CEA level was around 2.0 μg/L in patients without tumor

Table 1 Serum CEA and TPA-M levels by postoperative year (mean ± SD)

	Recurrence of neoplasia	1st yr	2nd yr	3rd yr	4th yr	*P*
CEA (μg/L)	-	1.5 ± 0.9	2.0 ± 1.8	1.4 ± 1.1	1.9 ± 1.0	< 0.01
	+[b]	6.9 ± 9.8	66.3 ± 130.8	19.9 ± 32.0	1.2 ± 0.7	
TPA-M (U/L)	-	64.4 ± 47.8	50.8 ± 38.4	54.0 ± 19.5	43.6 ± 29.0	< 0.05
	+[a]	192.2 ± 328.8	442.7 ± 652.8	93.3 ± 82.0	343.5 ± 437.6	

[a]$P < 0.05$, [b]$P < 0.01$, *vs* no recurrence.

recurrence and around 66.3 μg/L in those with recurrence with a sensitivity of 50% and a specificity of 76.9% and an accuracy of 67.9%. The mean level of circulating TPA-M was around 50.8 U/L in the patients without tumor recurrence and around 442.7 U/L in those with a recurrence, with sensitivity of 75%, a specificity of 71.8% and an accuracy of 78.2%.

Various investigations have suggested that raised level of serum CEA is an important indicator for the recurrence of colorectal neoplasia[4,7]. However, it is not unanimous because of the lack of practical results obtained through clinical follow-up of patients[24]. Nonetheless, periodic postoperative assay of CEA is considered an effective low-cost measure for identifying tumor recurrence[25]. No studies have been undertaken to analyze the efficiency of cytokeratins in detecting recurrences originating from colorectal adenocarcinoma. The present study demonstrated that TPA-M and CEA were comparable in terms of sensitivity, specificity and accuracy in the detection of recurrences. Further studies are needed for evaluating and confirming the importance of TPA-M in following up patients with colon or rectum cancer after surgery.

According to the 2000 consensus of the American Society of Clinical Oncology[25], a postoperative finding of raised CEA concentration in peripheral blood indicates that closer endoscopic and radiological follow-up should be undertaken until such possible tumor recurrences are located. It is certain that patients with colorectal rumor recurrence are never completely cured. Such patients still carry neoplastic cells that are capable of developing when conditions are suitable.

The question is therefore whether intensive follow-up is effective. The medical literature presents conflicting opinions in this respect. One option is to follow up with assay of tumor markers in patients and additional tests if higher than normal concentrations are found[4]. Another alternative is to undergo periodic tests, such as radiological examinations and endoscopic procedures, for rapid identification of reoccurrences of neoplasia and prompt reintervention[6]. Nonetheless, no significant improvements have been achieved in patient survival obtained through close medical follow-up for recurrences of colorectal neoplasia. Minton *et al*[26] showed that reoperation can achieve a five-year disease-free survival. Moertel *et al*[24] carried out interventions in 417 patients with recurrence, and found that 2.3% of them have no relapse in more than one year. Lucha Jr. *et al*[7] achieved a five-year survival in three reoperated patients (1.1%) out of 285 who were

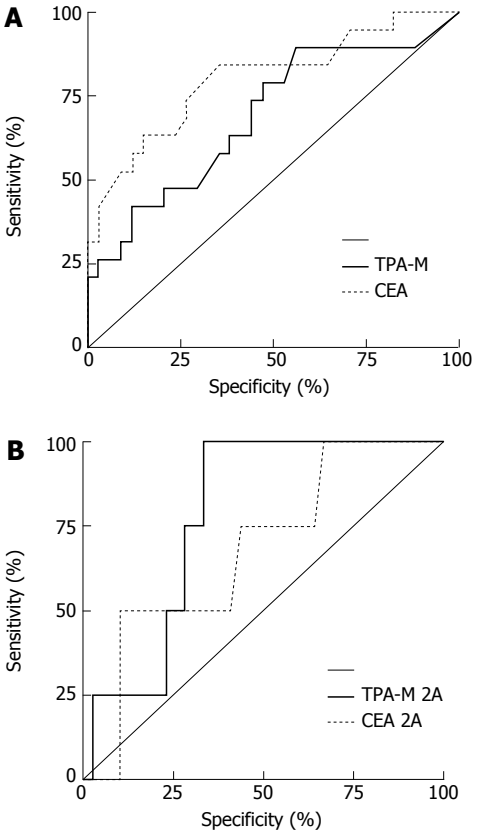

Figure 1 ROC curve for CEA and TPA-M in the first (**A**) and second (**B**) postoperative year among patients with colorectal cancer.

given radical treatment. None of the patients in the present study with recurrence of colorectal neoplasia after the initial resection could be cured. The tumor marker levels are increased in peripheral blood before recurrence of the neoplasia can be identified, which has been confirmed by a recent study[7]. Early surgical intervention at the site of tumor recurrence might become possible through improvements in the imaging methods available[4].

There are some possible explanations why the rates of curative resection of tumor recurrence are low. Sometimes, when resection of a metastasis in a given organ is carried out, the patient may still have micrometastases in other organs, and thus the surgery must be classified as non-radical. Institutional delays cause patients for reintervention with disease that is more advanced than it is when the recurrence is discovered, thereby always obtaining worse results. Moreover, metastases are often multiple, making it difficult to obtain complete resection

and thus compromise the cure for the patient.

Whether intensive follow-up prolongs patient survival is unclear. Ohlsson *et al*[27] evaluated 107 patients in Sweden who were divided into intensive and non-intensive follow-up groups after curative surgery for colorectal neoplasia, and found that there is no statistical difference in the two groups. Secco *et al*[28] followed up 216 patients with colon or rectum cancer undergoing curative surgery for lesion resection (127 of them had intensive follow-up), and found that adherence to a strict medical follow-up program can prolong survival of colorectal cancer patients. There is a consensus that the ideal marker for colorectal neoplasia does not yet exist[29].

Intensive follow-up in the present study enabled early detection of recurrences of colorectal neoplasia, through levels of the tumor markers CEA and TPA-M were increased, which even occurred before the recurrence site was located. Increased CEA and TPA-M levels prior to locating the recurrences occurred in 30% and 48% of the patients with recurrences. However, although reoperation was performed, no cure was obtained.

In conclusion, intensive follow-up by serial assay of CEA and cytokeratins allows early detection of colorectal neoplasm recurrence.

REFERENCES

1 **Bates SE**. Clinical applications of serum tumor markers. *Ann Intern Med* 1991; **115**: 623-638
2 **GOLD P**, FREEDMAN SO. DEMONSTRATION OF TUMOR-SPECIFIC ANTIGENS IN HUMAN COLONIC CARCINOMATA BY IMMUNOLOGICAL TOLERANCE AND ABSORPTION TECHNIQUES. *J Exp Med* 1965; **121**: 439-462
3 **Hünerbein M**. The value of tumor markers in colorectal cancer. *Recent Results Cancer Res* 1998; **146**: 48-55
4 **Carriquiry LA**, Piñeyro A. Should carcinoembryonic antigen be used in the management of patients with colorectal cancer? *Dis Colon Rectum* 1999; **42**: 921-929
5 **Fisher ER**, Sass R, Palekar A, Fisher B, Wolmark N. Dukes' classification revisited. Findings from the National Surgical Adjuvant Breast and Bowel Projects (Protocol R-01). *Cancer* 1989; **64**: 2354-2360
6 **Wiratkapun S**, Kraemer M, Seow-Choen F, Ho YH, Eu KW. High preoperative serum carcinoembryonic antigen predicts metastatic recurrence in potentially curative colonic cancer: results of a five-year study. *Dis Colon Rectum* 2001; **44**: 231-235
7 **Lucha PA Jr**, Rosen L, Olenwine JA, Reed JF 3rd, Riether RD, Stasik JJ Jr, Khubchandani IT. Value of carcinoembryonic antigen monitoring in curative surgery for recurrent colorectal carcinoma. *Dis Colon Rectum* 1997; **40**: 145-149
8 **Fuszek P**, Lakatos P, Tabak A, Papp J, Nagy Z, Takacs I, Horvath HC, Lakatos PL, Speer G. Relationship between serum calcium and CA 19-9 levels in colorectal cancer. *World J Gastroenterol* 2004; **10**: 1890-1892
9 **Kim SB**, Fernandes LC, Saad SS, Matos D. Assessment of the value of preoperative serum levels of CA 242 and CEA in the staging and postoperative survival of colorectal adenocarcinoma patients. *Int J Biol Markers* 2003; **18**: 182-187
10 **Fernández-Fernández L**, Tejero E, Tieso A. Significance of CA 72-4 in colorectal carcinoma. Comparison with CEA and CA 19-9. *Eur J Surg Oncol* 1995; **21**: 388-390
11 **Björklund B**, Björklund V. Specificity and basis of the tissue polypeptide antigen. *Cancer Detect Prev* 1983; **6**: 41-50
12 **Ishigami SI**, Arii S, Furutani M, Niwano M, Harada T, Mizumoto M, Mori A, Onodera H, Imamura M. Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer. *Br J Cancer* 1998; **78**: 1379-1384
13 **Polge A**, Bourgaux JF, Bancel E, Pignodel C, Boyer JC, Poirey S, de Bornier BM, Balmes JL, Bali JP. p53 and follow-up of colorectal adenocarcinomas. *Dig Dis Sci* 1998; **43**: 1434-1442
14 **Carpelan-Holmström M**, Haglund C, Lundin J, Alfthan H, Stenman UH, Roberts PJ. Independent prognostic value of preoperative serum markers CA 242, specific tissue polypeptide antigen and human chorionic gonadotrophin beta, but not of carcinoembryonic antigen or tissue polypeptide antigen in colorectal cancer. *Br J Cancer* 1996; **74**: 925-929
15 **Correale M**, Arnberg H, Blockx P, Bombardieri E, Castelli M, Encabo G, Gion M, Klapdor R, Martin M, Nilsson S. Clinical profile of a new monoclonal antibody-based immunoassay for tissue polypeptide antigen. *Int J Biol Markers* 1994; **9**: 231-238
16 **Plebani M**, De Paoli M, Basso D, Roveroni G, Giacomini A, Galeotti F, Corsini A. Serum tumor markers in colorectal cancer staging, grading, and follow-up. *J Surg Oncol* 1996; **62**: 239-244
17 **Lewenhaupt A**, Ekman P, Eneroth P, Nilsson B, Nordström L. Tissue polypeptide antigen (TPA) as a prognostic aid in human prostatic carcinoma. *Prostate* 1985; **6**: 285-291
18 **Panza N**, Pacilio G, Campanella L, Peluso G, Battista C, Amoriello A, Utech W, Vacca C, Lombardi G. Cancer antigen 125, tissue polypeptide antigen, carcinoembryonic antigen, and beta-chain human chorionic gonadotropin as serum markers of epithelial ovarian carcinoma. *Cancer* 1988; **61**: 76-83
19 **Gronowitz JS**, Bergström R, Nôu E, Påhlman S, Brodin O, Nilsson S, Källander CF. Clinical and serologic markers of stage and prognosis in small cell lung cancer. A multivariate analysis. *Cancer* 1990; **66**: 722-732
20 **Tizzani A**, Casetta G, Cavallini A, Piana P, Piantino P. [Blood and urine determinations of tissue polypeptide antigen in patients with bladder carcinoma]. *Minerva Urol Nefrol* 1990; **42**: 69-71
21 **Barak M**, Steiner M, Finkel B, Abrahamson J, Antal S, Gruener N. CA-15.3, TPA and MCA as markers for breast cancer. *Eur J Cancer* 1990; **26**: 577-580
22 **Fletcher RH**, Fletcher SW, Wagner EW. Epidemiologia Clínica. Porto Alegre: Artes Médicas, 1989
23 **Neter J**, Kutner MH, Nachtsheim CJ, Wasserman W. Applied Linear Statistical Models. 4th ed. Chicago: Irwin, 1996
24 **Moertel CG**, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen C. An evaluation of the carcinoembryonic antigen (CEA) test for monitoring patients with resected colon cancer. *JAMA* 1993; **270**: 943-947
25 **Bast RC Jr**, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG, Somerfield MR. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2001; **19**: 1865-1878
26 **Minton JP**, Hoehn JL, Gerber DM, Horsley JS, Connolly DP, Salwan F, Fletcher WS, Cruz AB Jr, Gatchell FG, Oviedo M. Results of a 400-patient carcinoembryonic antigen second-look colorectal cancer study. *Cancer* 1985; **55**: 1284-1290
27 **Ohlsson B**, Breland U, Ekberg H, Graffner H, Tranberg KG. Follow-up after curative surgery for colorectal carcinoma. Randomized comparison with no follow-up. *Dis Colon Rectum* 1995; **38**: 619-626
28 **Secco GB**, Fardelli R, Rovida S, Gianquinto D, Baldi E, Bonfante P, Derchi L, Ferraris R. Is intensive follow-up really able to improve prognosis of patients with local recurrence after curative surgery for rectal cancer? *Ann Surg Oncol* 2000; **7**: 32-37
29 **Fernandes LC**, Kim SB, Matos D. Cytokeratins and carcinoembryonic antigen in diagnosis, staging and prognosis of colorectal adenocarcinoma. *World J Gastroenterol* 2005; **11**: 645-648

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