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Preoperative serum CD26 levels: diagnostic efficiency and predictive value for colorectal cancer

OJ Cordero[1], D Ayude[2], M Nogueira[1], FJ Rodríguez-Berrocal[2] and M Páez de la Cadena[2]

[1]Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, Faculty of Biology, 15706 Santiago de Compostela; [2]Department of Biochemistry, Genetics and Immunology, University of Vigo, Faculty of Sciences, 36280 Vigo, Spain

Summary CD26 is an ectoenzyme with dipeptidyl peptidase IV activity expressed on a variety of cell types. Although the function of the high concentration of serum-soluble CD26 (sCD26) is unknown, it may be related to the cleavage of biologically active polypeptides. As CD26 or enzymatic activity levels were previously associated with cancer, we examined the potential diagnostic and prognostic value of preoperative sCD26 measurements by ELISA in colorectal carcinoma patients. We found a highly significant difference between sCD26 levels in healthy donors (mean 559.7 ± 125.5 μg l^{-1}) and cancer patients (mean 261.7 ± 138.1 μg l^{-1}) ($P < 0.001$). A cut-off at 410 μg l^{-1} gave 90% sensitivity with 90% specificity which means that the diagnostic efficiency of sCD26 is higher than that shown by other markers, particularly in patients at early stages. Moreover, sCD26 as a variable is not related with Dukes' stage classification, age, gender, tumour location or degree of differentiation. With a follow-up of 2 years until recurrence, preliminary data show that sCD26 can be managed as a prognostic variable of early carcinoma patients. In addition, the origin of sCD26 is discussed. © 2000 Cancer Research Campaign

Keywords: colorectal cancer; serum CD26; CD26/DPP-IV; diagnosis; tumour recurrence

Malignant transformation from normal to cancerous tissue is associated with cell-surface glycoprotein and glycolipid modifications (Hakomori, 1989). These glycoconjugates can be released in the circulation through increased cell turnover, secretion or shedding from the malignant cells and have been considered as potential tumour markers for helping in screening, diagnosis, staging, prognosis and monitoring of cancer therapy (Cohn et al, 1986). The protease dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5.) is a 110 000 MW cell-surface glycoprotein expressed on a variety of cell types, particularly melanocytes, epithelial cells (Iwata and Morimoto, 1999) and lymphocytes, where DPP-IV is necessary for normal immune function (see review, De Meester et al, 1999) and was assigned to the CD26 cluster. CD26 is a functional receptor for collagen (Bauvois, 1988; Dang et al, 1990) and was also recently identified as the adenosine deaminase binding or complexing protein (ADAbp, ADCP) (Kameoka et al, 1993; De Meester et al, 1994). Significant levels of DPP-IV activity have been shown to exist in plasma, serum and urine (Sharpé et al, 1988). The MW of serum CD26 (sCD26) suggests that it is originated by a shedding of membrane CD26 (Iwaki-Egawa et al, 1998). sCD26 can cleave NH_2-terminal dipeptides from polypeptides with either L-proline or L-alanine at the penultimate position (Fleischer, 1994). Many biologically active polypeptides have this sequence, for example substance P, chorionic gonadotropin, monomeric fibrin, promellitin (Bauvois et al, 1992) and regulatory peptides such as glucagon-like peptides 1 and 2 (Drucker et al, 1997). A proline residue is also present at the P1 position in many cytokines, such as IL-1β, IL-2, IL-6, and G-CSF (Ansorge et al, 1991). CD26 enzymatic activity also affects TNF-α (Bauvois et al, 1992), neuropeptides such as Y and YY (Medeiros and Turner, 1994) and chemokines activity (Oravecz et al, 1997; Proost et al, 1998).

CD26, when known as ADCP, was described to be consistently associated with cancer. The loss of DPP-IV expression during malignant transformation has been best characterized in melanocytic cells, although a role for DPP-IV in regulating the malignant phenotype had not been shown until very recently (Iwata and Morimoto, 1999; Wesley et al, 1999). A deficiency in solubilized CD26 was reported in total homogenates of tumours of colon, kidney, lung and liver (Ten Kate et al, 1985; 1986*a*), as well as in different transformed or cancer-derived cell lines (Ten Kate et al, 1986*b*). On the contrary, cell-surface CD26 expression has been correlated with disease aggressiveness of T and B cell lymphomas and leukaemias, follicular cell-derived thyroid carcinomas and basal cell carcinomas (reviewed in Iwata and Morimoto, 1999). In addition, serum DPP-IV activity was increased in patients with hepatic cancer (Hino et al, 1975; Kojima et al, 1987), and decreased in patients with blood, solid and oral (Fujita et al, 1977; Mogi et al, 1986; Uematsu et al, 1996) cancer. From these data, it seems helpful to fully evaluate the potential significance of serum CD26 as a colon carcinoma (which remains a major medical problem) (American Society of Clinical Oncology, 1996; 1998) diagnostic and prognostic marker.

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Correspondence to: OJ Cordero

MATERIALS AND METHODS

Patients

Preoperative blood and primary tumour samples were collected between January 1994 and December 1997 from 110 potentially curable patients (56 females, 54 males, mean age 68, range 34–88), operated for colorectal cancer (74 colon, 36 rectum) in the

Complexo Hospitalario Xeral-Cíes, Vigo, Spain. Twenty-three patients from the same hospital with other diseases were also studied: nine patients with gastrict tract carcinomas, five patients with Crohn's disease, five patients with benign pathology of the gastric tract and four patients with blood cell cancer. The control group, consisting of 52 healthy blood donors (23 females, 29 males) was provided by the Centro de Transfusion de Galicia throughout 1997. Some data of the population samples came from other studies (Fernández-Rodríguez et al, 2000; Ayude et al, 2000).

For prognostic studies, no patient receiving adjuvant therapy either preoperatively or postoperatively was included. Patients with family adenomatous polyposis coli, inflammatory bowel disease, or previous colorectal cancer were not included for review. The presence of metastasis or the failure to resect all the tumour deemed the resection palliative and these patients were excluded from analysis, as were those who died within 30 days of surgery. Thus, only 87 potentially cured patients with Dukes' stages A–C were followed-up for 2 years until recurrence.

Preparation of samples

The drawn blood was allowed to coagulate at room temperature and centrifuged at 2000 ***g*** for 15 min. The sera were stored at –85°C until used. All adenocarcinoma samples were processed for regular pathological and histological examination.

Immunoassays

The concentration of serum CD26 and carcinoembryonic antigen (CEA) were analysed using specific immunoassays (human soluble CD26 ELISA Kit from Bender Medsystems, Vienna, Austria, and Enzymun-Test CEA from Boehringer Mannheim, Germany). ELISAs were performed according to the manufacturer's instructions: mean values of duplicated measurements were calculated and a sigmoid-shaped standard curve was determined by simultaneously analysing a dilution series of standard samples. Specificity of sCD26 system was evaluated by the manufacturer for several circulating factors of the immune system and no cross-reactivity was detected. No cross-reactivity of anti-sCD26 Abs with rheumatoid factor was found in a previous study (manuscript submitted).

Prognostic predictors

Five clinical and pathological variables were evaluated according to the following definitions: age, sex, tumour location, stage, and degree of differentiation. Age was recorded in years at time of operative intervention; for statistical analysis, patients were grouped into two categories, ≤ 50 and > 50 years old. For the recurrence study, the cut-off chosen was ≤ 76 or > 76, to better discriminate the two groups. Recurrence pattern based on patients' sex was also analysed. The site of the primary lesion was determined from the operative report. The large-bowel was divided into two regions for statistical analysis: colon (including right and left colon, and sigma) and rectum (lesions were considered rectal if their origin was below the peritoneal reflection). The stage of disease was originally reported using Dukes' classification (invasive and metastatic potential of tumours) (Dukes, 1932) as determined in the original operation. The degree of differentiation as described by the pathologist in the original operation was recorded and classified into three categories: well, moderately and poorly differentiated lesions.

Statistical methods

Normal distribution was assessed using the Kolmogorov-Smirnov test. Variance homogeneity was evaluated by the Levene test. The statistical significance of the results was assessed using a nonpaired Student's *t*-test or Mann–Whitney U test performed in the SPSS program for Windows (Release 7.5.2S, or 8.0). Statistical comparisons among groups were made by Kruskal–Wallis and ANOVA tests, according to the Levene test results, respectively. A cut-off value for CD26 was determined using receiver operating characteristics (ROC). ROC curves are plots of the percentage true-positives (sensitivity) against the percentage false-positives (100-specificity) for multiple thresholds (Beck and Shultz, 1986; Zweig and Campbell, 1993). In order to evaluate the impact of each variable over the disease-free interval, a postoperative follow-up of the patients was performed. Kaplan–Meier curves (Kaplan and Meier, 1958) were constructed, with colorectal cancer-related recurrence as the primary end-point. Differences in disease-free survival (DFS) among groups were assessed by log-rank analysis. P values ≤ 0.05 were considered statistically significant.

RESULTS

sCD26 levels in serum of healthy donors and patients with colorectal cancer

Soluble CD26 concentration (μg l^{-1}) was determined in 110 sera from patients with colorectal cancer and 52 control sera from healthy donors. Data from both populations follow a normal distribution. The concentration of sCD26 was dramatically impaired in many colorectal cancer patients (261.65 ± 138.07 μg l^{-1}, range 56–980 μg l^{-1}) with respect to control donors (559.65 ± 125.52 μg l^{-1}, range 273–863 μg l^{-1}), on average by 53% ($P < 0.001$).

Statistical analysis after this comparison gave the results shown in Table 1. There were no significant differences between the two sex- and age-groups for both donor and patient samples, which is particularly interesting in the second case, due to the strong difference between donor and patient samples in the number of recruited individuals for both age-groups. These data agree with the fact that DPP-IV activities did not differ significantly with age (Hino et al, 1975).

Relationship between preoperative serum sCD26 levels and clinicomorphologic features of tumours

Table 2 compares by the Kruskal–Wallis test preoperative serum sCD26 levels and the Dukes' stages from patients with colorectal adenocarcinoma. In addition, statistical comparisons between groups were made by using the Mann–Whitney U test, as there was not variance homogeneity. By these analyses, there is no difference in the preoperative serum activity of sCD26 among Dukes' stages. The possible association between preoperative serum sCD26 levels and age, sex, tumour location and degree of histologic differentiation of tumours was also examined. According to our data (Tables 1, 2 and 3) none of these properties was correlated with preoperative serum sCD26 levels.

Table 1 Serum CD26 concentration in donors and patients with colorectal cancer

Case	*n*	Mean ± SD (μg l^{-1})	SEM	Range	Student's *t*-test
Donors	**52**	**559.65 ± 125.53**	**17.41**	**273–863**	
Sex					
Men	23	584.70 ± 113.93	23.76		
					NS
Women	29	539.79 ± 132.58	24.62		
Age					
< 50 years	39	557.36 ± 126.31	20.23		
					NS
≥ 50 years	13	566.54 ± 127.95	35.49		
					***P* < 0.001**
Tumoural	**110**	**261.65 ± 138.07**	**12.45**	**56–980**	
Sex					
Men	56	235.77 ± 101.71	13.59		
					NS
Women	54	273.52 ± 155.92	21.22		
Age					
< 50 years	9	218.56 ± 77.42	25.81		
					NS
≥ 50 years	101	257.49 ± 135.50	13.48		

NS = not significant; *P* = statistical significance. The age cut-off point (50 years) was chosen because it can be used in both donor and tumoural groups, facilitating comparison

Table 2 Relationship between the levels of sCD26 and the Dukes' stage classification

Dukes' stage	*n*	Mean ± SD (μg l^{-1})	SEM	Median	Range	Kruskal–Wallis test
A	12	295.33 ± 152.34	43.98	292	78–663	
B	55	226.67 ± 93.06	12.55	205	100–438	NS
C	29	251.48 ± 95.45	17.73	248	78–522	
D	14	333.50 ± 243.10	64.97	312	56–980	

NS = not significant

sCD26 levels in serum of patients with other related carcinomas and benign diseases

The level of sCD26 was also determined in serum of 23 patients with related diseases other than colorectal cancer, preliminarily to check the specificity of our finding. These patients were included in three different groups: patients with gastric-tract carcinomas (GC, $n = 9$), patients with blood cell cancer (BCC, $n = 4$) and patients with Crohn's disease or with benign pathology of the gastric tract (BPI, $n = 10$). Results shown in Figure 1 suggest that sCD26 levels discriminate well colorectal carcinoma from gastric (585 ± 148 μg L^{-1}) and blood cell cancer (663 ± 196 μg l^{-1}), and that a study on sCD26 in different BCCs could clear the origin of sCD26 because its levels are enhanced in some but not in other BCCs. Curiously, the majority of BPI show low sCD26 levels whereas Crohn's patients presented a very irregular distribution.

Diagnostic efficiency of sCD26 preoperative serum levels

Receiver operating characteristics (ROC) curves for sCD26 and CEA are represented in Figure 2. The corresponding sensitivities at different specificity levels for sCD26 are provided in Table 4. The ideal cut-off point for diagnostic value of sCD26 was determined by random selection of outstanding points from the ROC curve. The best efficiency (90%) was obtained with the point 410 μg l^{-1}, representing a sensitivity of 90% with a specificity of 90%. Even with a specificity of the 100% the efficiency is good (71%). This curve revealed that the diagnostic efficiency of sCD26 levels is higher than that shown by CEA, the more extensively used in health services, which showed an efficiency of the 69%. We also studied the diagnostic efficacy of sCD26 in Dukes' stages A, B, C and D patients. Figure 3 shows that the sensitivity is enhanced in the A, B and C stages, whereas was impaired in the Dukes' stage D, in which CEA levels diagnosed better.

sCD26 as prognostic predictor in colorectal cancer

From the 110 patients with colorectal adenocarcinoma included in this work, only 87 potentially cured patients with Dukes' stages A–C were followed-up for at least 1 year or until recurrence. After a mean postoperative follow-up period of 22 months, recurrence appeared in 18 patients. Disease-free survival time was 39 months (mean confidence interval, 35–43, maximum 49, $n = 87$) and the percentage of tumoural recurrence was 21.1%. The survival curve of the total potentially cured patients included in this study is plotted in Figure 4A. The same study was performed considering only patients in Dukes' stage B (Figure 4B) or C (data not shown) (there was not enough data for A), finding a non-significant result and a particularly bad behaviour of sCD26 as a prognostic marker in Dukes' stage C. Table 5 shows the results of a univariate survival analysis of patients stratified into groups by biochemical, clinical and pathological features. From these parameters, only the Dukes' stages, as currently described, and sCD26 were related to

Table 3 Relationships between preoperative sCD26 levels and the degree of tumour differentiation or location

	n	Mean ± SD (μg l^{-1})	SEM	ANOVA
Degree of differentiation				
Good	10	257.30 ± 86.28	27.28	
Moderate	90	247.83 ± 112.92	11.90	NS
Poor	9	235.00 ± 141.82	47.27	
Location				**Student's *t***
Colon	74	251.24 ± 141.69	16.47	
				NS
Rectum	36	260.58 ± 110.69	18.45	

NS = statistically not significant. With regard to the degree of differentiation, information from one patient was lost

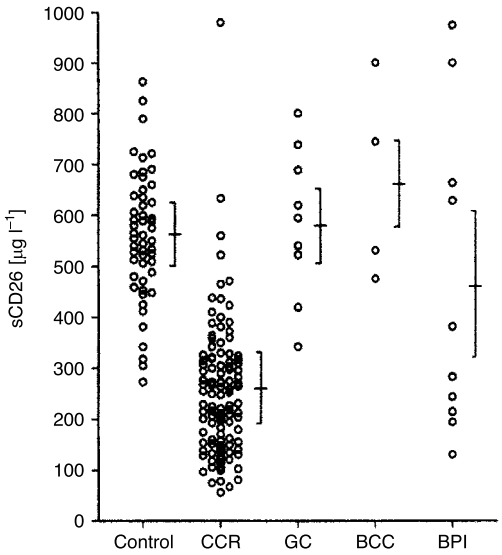

Figure 1 Serum CD26 (μg l^{-1}) in healthy donors and patients with: colorectal cancer (CRC), gastric-tract carcinoma (GC), Crohn's disease and other benign pathology of intestinal tract (BPI), and blood cell cancer (BCC). The vertical lines represent the SD values

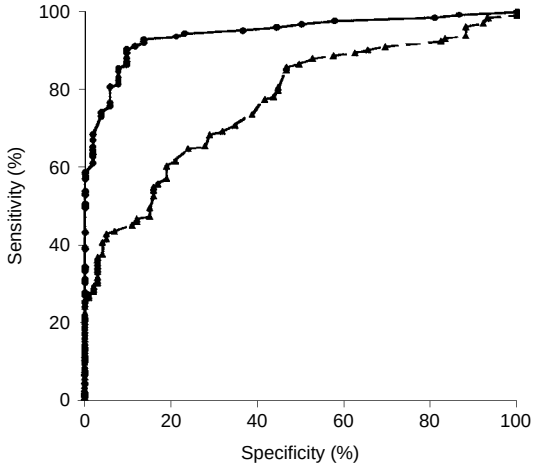

Figure 2 ROC curves for the serum levels of CD26 and CEA

long-term outcome in this study. For sCD26, two groups of patients could be differentiated choosing the cut-off in the 60th percentile (250 μg l^{-1}), the log-rank test giving a $P = 0.0346$. Data from the survival curves of Figure 4 and the fact that age and degree of differentiation (Table 5) are near to significance in this work, are encouraging us to study a larger follow-up period.

DISCUSSION

Cell-surface proteases participate in malignant transformation and cancer progression by facilitating invasion and metastasis. However, they may also have the opposite effect. This is the case for DPP-IV/CD26 (Werb, 1997; Iwata and Morimoto, 1999). The diverse biological functions of CD26 (De Meester et al, 1999; Iwata and Morimoto, 1999) may be responsible in part for the different roles of CD26 in various clinical settings. In melanoma cells, where membrane expression of DPP-IV is, as well as in CRC, lost or altered (Ten Kate et al, 1986*a*; Morrison et al, 1993), it has been demonstrated that the inducible translation of CD26 reverses the malignant phenotype. It is suggested that DPP-IV enzymatic activity degrades growth factors (unknown autocrine factors, although candidates should be among those cited in the introduction) required for survival of tumour cells.

Surprisingly, little information is available about the physiological activity of sCD26 in spite of its presence at relatively high concentrations in serum (~ 600 μg l^{-1}) of healthy donors. Studies like this should unravel the kind of cells which shed membrane CD26 to the serum. For example, as membrane CD26 is lost in hepatocellular carcinoma (Ten Kate et al, 1985; Iwata and Morimoto, 1999; Perner et al, 1999) and DPP-IV is increased in patients with hepatic cancer (Kojima et al, 1987) as well as in many studies of hepatic regeneration, liver epithelia is one of the suggested sCD26 sources. In this case, sCD26 levels would correlate with cell proliferation. This is clearly not the case for colorectal carcinoma as well as, our data suggest, for other pathologies such as the GC group. The studies on the membrane CD26 in human CRC (Ten Kate et al, 1985; 1986*b*) found a loss of expression in only 11% of the patients, and decreases in a third of the patients. Although this and our study, in which 58% of patients had lower values than the minimum range of normal donors, cannot be directly correlated, it is easily deduced that loss of membrane CD26 and enhancement of sCD26 are not correlated in

Table 4 Comparison of three cut-off points for sCD26 (μg l^{-1}) in the detection of colorectal cancer

Cut-off points	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Efficiency (%)
270 μg l^{-1}	59	100	100	50	71
330 μg l^{-1}	80	94	97	67	85
410 μg l^{-1}	90	90	96	80	90

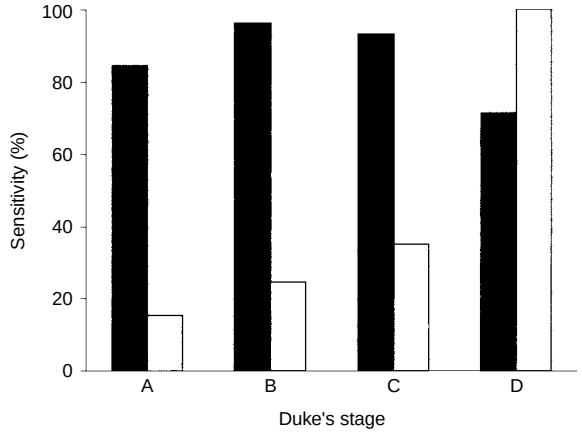

Figure 3 Sensitivity of sCD26 and CEA serum levels for the diagnosis of colorectal cancer by Dukes' stages

CRC. In addition, we found a lack of any direct correlation of sCD26 with tumour location, degree of histologic differentiation, kind of metastasis or Dukes' stages. In conclusion from all these data, 53% impairment in sCD26 in colorectal cancer does not seem to be originated by alteration of CD26 on CRC tumour cells.

At least two possibilities arise from this conclusion: that CRC, as well as BPI but not GC, is associated with an impairment of the usual hepatic function, or the drop in sCD26 levels is related to the immune system status. For the first case, no data have been published up to now. In fact, as CRC metastases were each all located in liver, an improvement in sCD26 levels should be expected, as well as a correlation with the kind of metastasis. However, a cross-talk between the lymphoid lineage and malignant tumours in vivo have been long discussed (Shibuya-Saruta et al, 1996; Gruss et al, 1997; Nano et al, 1997; Iwata and Morimoto, 1999) and some data about the immune defective antitumour response in CRC have been described before, including a defect in IL-12 production (O'Hara et al, 1998), which is a well-known CD26 up-regulator (Cordero et al, 1997) on T cells. In oral cancer patients, in which around a 50% decrease in serum DPP-IV activity has been reported, a correlation between sCD26 and CD26+ T was found, and the number of T lymphocytes and PBL and the amount of CD26 in T lymphocyte plasma membranes were significantly less than in healthy subjects (Uematsu et al, 1996; 1998). Both possibilities can explain the donor-dependent variations, but the second one seems more probable in the cases of hemicolectomy or rectoragy (some of the lower values of the BPI group), except the Dukes' stage D cases with high sCD26 values, perhaps associated with a high proliferative (hepatic) cellular state, or with a later activation of PBL as suggested in CRC and ovarian tumours for the soluble CD44v6 glycoprotein (Sliutz et al, 1995; Yamane et al, 1999). It seems, then, necessary to collect the lymphocyte count and other immune parameters of the patients in future studies of CRC. As some studies are showing that sCD26 therapy enhances the immune function in some pathological conditions such as AIDS (Schmitz et al, 1996), it might be interesting to analyse if CRC patients may well benefit from exogenous sCD26 treatment.

Very recently, the clinical utility of serum DPP-IV/CD26 activity measurements was tested in adult and paediatric patients with hepatobiliary diseases and in liver transplant recipients. The results established elevated serum DPP-IV activity as a clinically useful marker of cholestasis and demonstrate that DPP-IV levels do not change in metastatic bone disease (Lakatos et al, 1999; Perner et al, 1999) nor in allergic asthmatics, inhaling glucocorticoids or not (Van Der Velden et al, 1999). Higher activities were also found in serum of patients with osteoporosis, probably related to its severity (Gotoh et al, 1988). However, reduced peptidase can be found in healthy smokers (Van Der Velden et al, 1999), alcohol-dependent (Maes et al, 1999) and major depressive (Maes et al, 1997) donors. These last drops are also in agreement with an impaired immune response. The studies described before show alterations in the levels of the serum DPP-IV enzymatic activity. To our knowledge, only one study reported a decrease of protein sCD26 (but to a lesser extent than ours) in oral cancer patients (Mogi et al, 1986), although the authors measured a higher quantity of protein than we by using the commercial kit. In addition, some of our data are not in accordance with published measurements of DPP-IV enzymatic activities in some diseases such as GC (lower levels than in normal subjects) (Hino et al, 1975), or in some patients of the BCC group (Fujita et al, 1977). As at least one different serum protein accounting for the DPP-IV activity (Duke-Cohan et al, 1996) as well as new discovered cellular proteins with DPP-IV activity (Pangalos et al, 1999; Underwood et al, 1999) have been described, these facts should encourage more complete studies at the protein level on different pathologies. Therefore, although we found a normal distribution for sCD26 levels in both samples of Spanish populations, which points to a unique locus for the CD26 gene, a possible effect of race on the absolute levels detected in this study cannot be excluded.

Establishing the diagnosis at an early stage in colorectal cancer, with a simple biochemical index, is a current subject of research in clinical oncology. The CEA levels are the marker of reference in this neoplasia, although not recommended as diagnostic test for CRC (American Society of Clinical Oncology, 1996). Our curve revealed that sensitivities of sCD26 were higher at different specificity levels than those of CEA, as well as efficiency. Moreover, when the diagnostic sensitivity of sCD26 and CEA by Dukes'

Table 5 Univariate analysis of the disease-free survival (DFS) according to the classical clinicopathological and biochemical features of this study

Factor	Category	*n*	DFS (months)	Tumoural recurrence (%)	Log-Rank test (*P*)
Age					
	≤ 76 years	67	41.30	16.42	0.0561
	> 76 years	20	29.55	35.00	
Sex					
	Male	47	33.49	19.15	0.8103
	Female	40	39.82	22.50	
Location					
	Colon	59	39.93	16.95	0.1383
	Rectum	28	32.35	28.57	
Degree of differentiation					
	Good	5	29.98	20.00	
	Moderate	76	39.54	19.42	0.0820
	Poor	6	18.85	50.00	
Dukes' stage					
	A	7	–	0	
	B	52	40.57	17.31	0.0299
	C	28	25.33	32.14	
sCD26					
	≤ 250 μg l^{-1}	52	33.73	26.92	0.0346
	> 250 μg l^{-1}	35	38.97	11.43	

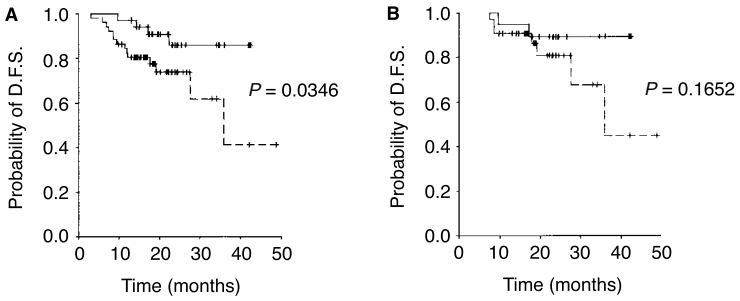

Figure 4 Kaplan–Meier recurrence curves for colorectal cancer patients stratified by the preoperative serum CD26 levels in (**A**) total patients, and (**B**) patients of Dukes' B. Group1 (—) = Patients with sCD26 above 250 μg l^{-1}. Group 2 (---) = Patients with sCD26 equal or below 250 μg l^{-1}

stage were compared, sCD26 presents higher sensitivity than CEA to diagnose patients in Dukes' A, B and C stages. As recently reported, serum TIMP-1 (plasma tissue inhibitor of metalloproteinase) or sCD44v6 screened better only the Dukes' stage D CRC (Holten-Andersen et al, 1999; Yamane et al, 1999), and serum YKL-40 (Cintin et al, 1999) was clearly a poorer marker of diagnosis. Our conclusion is that preoperative sCD26 level is an useful, easy to handle marker for early detection of potentially curable CRC.

Reported recurrence rates after curative resection of large-bowel adenocarcinoma varied widely, partly because of how a recurrence is defined (Stipa et al, 1991), from 3% to 50%, usually within 2 years of surgery. As the risk factors commonly identified (level of invasion, lymphatic involvement, and site of original carcinoma) (Michelassi et al, 1990) do not always allow prediction of the outcome, which may guide the physician in aggressive but more selective adjuvant therapy and targeted surveillance in follow-up (Obrand and Gordon, 1997), we also studied if CD26 can help to distinguish CRC cases at high risk of tumour recurrence. Two groups of patients were differentiated by placing a cut-off point at the 60th percentile (log-rank test P = 0.0346). According to our data there is no relationship between the preoperative serum sCD26 levels and the classical clinical features (Devesa et al, 1988). Meanwhile serum CEA levels, for example, correlate with the degree of histologic differentiation and the Dukes' stages classification. Thus new information about the prognosis of the patients is obtained. The different behaviour of sCD26 values in prognosis of Dukes' groups B and C can be also explained by the hypothesis explained above, because in C, the patients with an activation of the immune system (and thus with an

enhancement in their sCD26 levels) might show a better survival than those with lower sCD26 values. The fact that analysis was performed in 2 years of recurrence, that the measure is not related with the pathological stage and can be carried out before the surgical operation, appears nevertheless to justify a follow-up of sCD26 as a prognostic variable.

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