Prognostic Significance of Adiponectin Levels in Non-metastatic Colorectal Cancer

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Abstract. *Background: Circulating adiponectin levels are inversely correlated with the risk of colorectal cancer (CRC). This study was designed to evaluate the association between adiponectin levels and the clinicopathological variables of CRC and to analyze the possible prognostic value of adiponectin in predicting relapse-free survival. Patients and Methods: Baseline adiponectin and serum tumor markers were analyzed in 60 patients with non-metastatic CRC followed-up from time of surgery for at least three years or until relapse. Results: The median adiponectin levels were lower in CRC patients (8.3 μg/ml) than controls (13.1 μg/ml, p<0.001). Moreover, median adiponectin concentration gradually decreased with increase in tumor stage. Low pre-surgical adiponectin levels were found in 52% of the relapsing patients compared to 26% (p=0.037) of the non-relapsing patients. Logistic regression analysis demonstrated that stage of disease (OR (odds ratio)=15.9, p<0.01) and low adiponectin levels (OR=4.66, p<0.05) were independent predictors of recurrent disease. Conclusion: Low serum adiponectin might represent an adjunctive tool in risk prediction for CRC recurrence.*

The associations between excess weight, obesity and cancer, as well as the biological mechanisms contributing to these associations, are an evolving and very active area of research. It is generally accepted that endocrine dysfunction of adipose tissue may represent one of the causal links between obesity and cancer. Indeed, recent studies have indicated that some adipokines may significantly influence the growth and proliferation of tumor stroma and the malignant cells within (1). Among them, adiponectin is perhaps the most interesting and promising for the clinician, since it has a profound protective effect on the pathogenesis of obesity-related disorders (2).

Adiponectin is a 30-kDa protein hormone and cytokine secreted mainly by adipocytes, structurally related to the collagen superfamily and sharing homologies with collagens, complement factors and tumor necrosis factor-α (TNF-α) (3-6). Epidemiological studies have suggested that circulating adiponectin levels are inversely correlated with the risk of colorectal cancer (CRC) associated with obesity and insulin resistance (7, 8). More recently, a case-control study has demonstrated that men in the highest adiponectin quintile had an approximately 60% reduced risk of colorectal cancer compared to those in the lowest quintile, the association being independent of body mass index (BMI), waist circumference, waist-to-hip ratio and physical activity (9). Moreover, decreased concentrations of plasma adiponectin have been associated with the development of colon adenomas in Japanese patients (10). In particular, significant associations were found between decreased adiponectin levels and the number or size of tumors and histological progression from tubular to tubulovillous/villous adenomas (10).

Several mechanisms have been proposed that may link adiponectin to carcinogenesis, such as indirect effects through altering hormone and cytokine levels or direct effects through altering signal pathways involved in cell growth and proliferation (11). Furthermore, experimental

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evidence suggests that adiponectin is a direct angiogenesis inhibitor that preferentially induces apoptosis in activated endothelial cells in pathological neovascularization (12).

Previous studies have shown that circulating adiponectin levels were decreased in patients with gastric cancer (13) and the association of low adiponectin concentrations and CRC risk has been previously reported (7-9). No data are currently available on the distribution of adiponectin levels in CRC patients not on their prognostic significance. Therefore, the aim of the present study was to evaluate the possible associations between adiponectin and the clinicopathological variables of non-metastatic CRC patients at the time of diagnosis of a primary tumor. Moreover, a follow-up study was performed to analyze the possible prognostic value of pre-surgical adiponectin levels in predicting the disease-free survival of patients with CRC.

Patients and Methods

Patients. Sixty consecutive patients (31 males, 29 females, mean age 64±10 years) with histologically diagnosed non-metastatic primary colorectal adenocarcinoma (Dukes' stage A: n=7, stage B: n=34, stage C: n=19), treated at the Department of Surgery of the University of Rome "Tor Vergata", were enrolled into the study. All patients underwent surgical resection with curative intent. The clinical features of the CRC patients are summarized in Table I. As a control group, in a 2:1 ratio, 30 subjects (13 males, 17 females; mean age 59±12, ranging from 37 to 80 years) were also evaluated. Diabetes mellitus (fasting blood glucose level >115 mg/dL or treatment with a hypoglycemic agent), body mass index >28, history of alcohol or drug abuse, impaired liver (bilirubin level>1.5 mg/dl) or renal (creatinine level>1.5 mg/dl) function and a Karnofsky performance status lower than 90% were considered as exclusion criteria.

The patients were followed from the time of diagnosis of the primary tumor for at least 3 years after surgery or until time of recurrence (the median follow-up was 37 months, ranging from 3.9 to 69.1). All patients were generally reviewed at 3-month intervals during the first 2 years after surgery. Thereafter, the interval between visits increased to 6 or 12 months in relation to tumor stage. No patient was lost at follow-up. The study was performed under the appropriate institutional ethics approvals and in accordance with the principles embodied in the Declaration of Helsinki. Informed consent was obtained from each participating subject.

Sample collection and immunoassay. Blood samples from the CRC patients were drawn during the week before surgery, or prior to neoadjuvant chemotherapy and/or irradiation. After an overnight fast and a rest of at least 20 minutes, blood was drawn from each consenting subject by venipuncture of the antecubital vein using a 20G needle. Blood was allowed to clot and then centrifuged at 2000 g for 10 minutes at 4˚C. Serum samples were aliquoted, coded and stored at –80˚C until the assays were performed.

Serum adiponectin levels were determined by a commercially available enzyme immunoassay (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic) according to the manufacturer's instructions. Intra- and inter-assay co-efficients of variation were below 5% and 10%, respectively. The minimum detectable dose was 0.2 μg/ml.

Table I. *Clinical features of non-metastatic colorectal cancer patients. Comparison between relapsing and NED (no evidence of disease).*

Variable		Whole series N=60	NED N=35	*p*-value	Relapsing N=25
Age (years)	Mean±SD	64±10	63±9	NS	64±11
	Range	36-80	36-80		36-80
Males	N (%)	31 (52)	18 (51)	NS	13 (52)
Site of primary tumor	N (%)			NS	
Colon		15 (25)	9 (26)		6 (24)
Sigma		14 (23)	11 (31)		3 (12)
Rectum		31 (52)	15 (43)		16 (64)
Grading	N (%)			NS	
1		7 (12)	5 (14)		2 (8)
2		37 (61)	21 (60)		16 (64)
3		16 (27)	9 (26)		7 (28)
Dukes' stage	N (%)			0.004	
A		7 (12)	7 (20)		0 (0)
B		34 (56)	22 (63)		12 (48)
C		19 (32)	6 (17)		13 (52)
Total		60	35		25
Length of follow-up (Months)	Median (range)		42.6 (30.9-69.1)	<0.001	14.6 (3.9-43.0)
Site of recurrence					N (%)
Locoregional					6 (25)
Distant metastasis					19 (75)

Serum carcinoembryonic antigen (CEA) levels were measured using a commercially available immunoassay (Abbott Labs, Chicago, IL, USA). CA 72-4 and CA 19-9 levels were determined using the CA 72-4 DDRIA and the CA 19-9 RIA Kits (both by Fujirebio Diagnostics Inc., formerly Centocor Diagnostics, Malvern, PA, USA). The cut-off limits chosen for sample evaluation were 5 ng/ml, 6 U/ml and 37 U/ml for CEA, CA 72-4 and CA 19-9, respectively.

Measurements were ascertained while blinded to the sample origin. All samples were assayed in duplicate and those showing values above the standard curve were re-tested with appropriate dilutions.

Statistical analysis. Differences between percentages were assessed by chi-square test. Student's unpaired *t*-test and Anova test were used for the normally distributed variables. Appropriate non-parametric tests (Mann-Whitney *U*-test and Kruskal-Wallis ANOVA and median test) were employed for all the other variables. Univariate and multivariate linear regression analyses were performed to assess the possible associations between adiponectin and the clinicopathological variables. Logistic regression analysis was used to evaluate the odds ratios (OR) for outcome (defined as recurrence) between high and low adiponectin groups. Data are presented as percentages, mean±SD, or median and interquartile ranges (IQR). Only *p*-values lower than 0.05 were

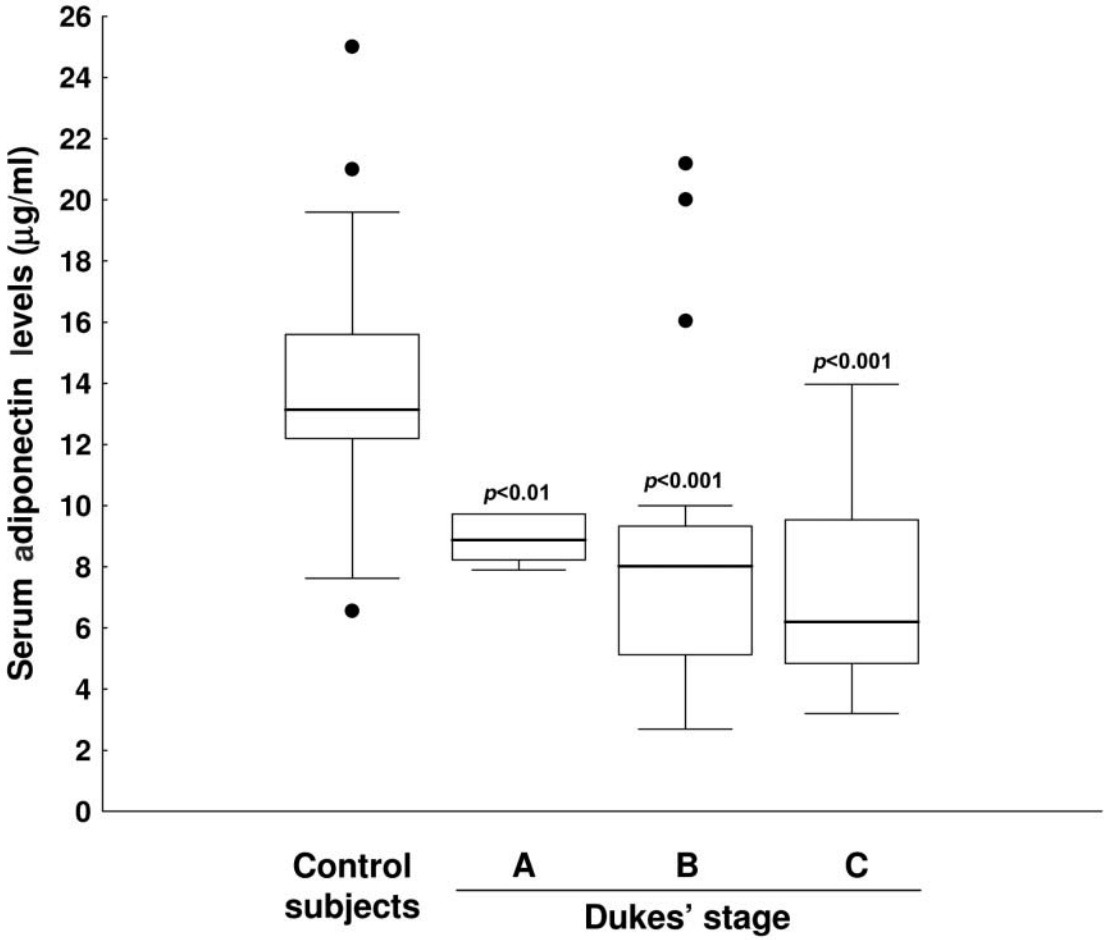


Figure 1. *Box-plot analysis of serum adiponectin levels in 60 non-metastatic CRC patients stratified on the basis of the stage of disease and 30 control subjects. Data are presented as median values (solid lines), interquartile range (columns) and non-outlier (whiskers) ranges. Closed circles indicate outliers. Mann-Whitney test of single stages CRC compared to control subjects.*

regarded as statistically significant. All calculations were made using computer software packages (EGRET Cytel Software Co., Cambridge, MA, Statistica, StatSoft Inc., Tulsa, OK, USA).

Results

The median serum adiponectin levels were lower in the CRC patients (8.3 (5.5-9.4) μg/ml) than in the control subjects (13.1 (12.2-15.6) μg/ml, $p<0.0001$). Moreover, as shown in Figure 1, the median adiponectin concentration gradually decreased with increase in tumor stage (Stage A: 8.9 (8.2-9.7) μg/ml; Stage B: 8.5 (5.8-9.3) μg/ml; Stage C: 6.2 (4.8-9.5) μg/ml), although the difference within stages did not reach the level of significance.

Serum adiponectin was then categorized into low (<6.4 μg/ml) or high (≥6.4 mg/ml) according to a cut-off value calculated from the mean –2SD of the values observed in the control subjects (14.2±3.9 μg/ml). The associations between adiponectin and the clinicopathological variables were analyzed after categorization. As shown in Table II, no significant correlation was observed between the serum adiponectin level and the site of the primary tumor or grading. In the male patients, 18 (58%) out of 31 showed low adiponectin levels compared to four (14%) out of 29 of the female patients ($p<0.001$). More interestingly, low adiponectin levels were found in approximately 35% and 53% of the patients with stage B and C of disease, whereas all the stage A patients had high serum adiponectin levels (Table II).

To assess the possible determinants of adiponectin among the clinical and laboratory features of CRC, a multiple regression analysis was performed in which adjustments were made for the following variables: age, sex, site of primary tumor, grading, serosal and lymph node involvement, Dukes' stage, CEA, CA 19-9 and CA 72-4 tumor markers. As shown

Table II. *Association between clinicopathological variables and serum adiponectin levels in non-metastatic colorectal cancer patients.*

Variable	N	Serum adiponectin levels* <6.39 μg/ml	≥6.39 μg/ml	*p*-value
Gender				
Male	31	18 (58.1)	13 (41.9)	
Female	29	4 (13.8)	25 (86.2)	<0.001
Site of primary tumor				
Colon	15	4 (26.7)	11 (73.3)	
Sigma	14	6 (38.7)	8 (61.3)	
Rectum	31	12 (42.9)	19 (57.1)	0.63
Grading				
1	7	3 (42.9)	4 (57.1)	
2	37	12 (32.4)	25 (67.6)	
3	16	7 (43.8)	9 (56.2)	0.69
Serosal involvement				
No	43	13 (30.2)	30 (69.8)	
Yes	17	9 (52.9)	8 (47.1)	0.10
Lymph node involvement				
N0	41	12 (29.3)	29 (70.7)	
N+	19	10 (52.6)	9 (47.4)	0.08
Dukes' stage				
A	7	0 (0.0)	7 (100)	
B	34	12 (35.3)	22 (64.7)	
C	19	10 (52.6)	9 (47.4)	0.05

*Categorized according to a cut-off value calculated from the mean value – 2SD of the values observed in the control subjects. Numbers in parentheses represent percentages.

in Table III, Dukes' stage of disease and male gender were both independent predictors for low adiponectin levels (R^2 for the entire model=0.28, $p<0.001$).

Clinical information on post-operative follow-up was available from all the CRC patients. Over a median follow-up period of 37 months, 35 (58%) out of the 60 patients remained free of disease, while 25 (42%) patients experienced relapsing disease (Table I). No differences were observed in age, sex, site of primary tumor or grading between patients with and without recurrence (Table I). Pre-surgical adiponectin levels were below the cutoff in approximately 52% of the relapsing patients compared to 26% ($p=0.037$) of the patients who remained free of disease. A multiple logistic regression analysis was performed to compute the odds ratios (ORs) and 95% confidence intervals (CI) for recurrent disease. As shown in Table IV, both Dukes' stage of disease (OR=15.9, $p<0.01$) and low adiponectin levels (OR=4.66, $p<0.05$) were independent predictors of recurrent disease. Figure 2A demonstrates the Kaplan-Meier disease-free survival curves for non-metastatic CRC patients with low (below the cut-off value) or high (above the cut-off value) adiponectin levels. As shown, low pre-surgical adiponectin levels were associated with an increased recurrence rate compared to patients with high levels of this adipokine (Log-rank statistic=2.11, $p=0.035$). Similar results were obtained when only Dukes' stage B CRC patients were included in the analysis (Log-rank statistic=1.94, $p=0.053$) (Figure 2B).

Discussion

The results obtained in this study indicate for the first time that serum adiponectin levels are significantly decreased in patients with non-metastatic CRC compared to control subjects, and that low circulating adiponectin might represent a risk factor for recurrent disease. It is currently recognized that nutritional status in patients with cancer might also affect adiponectin levels (14). However, the pre-surgical albumin, total protein, total cholesterol and triglyceride levels were not altered in the recruited patients (data not shown). Furthermore, patients with metastatic disease were excluded from the study to avoid any confounding variable linked to the presence of cancer cachexia, in which weight loss is significantly associated to increased adiponectin concentrations (14). All these considerations suggest that in this study the differences observed in serum adiponectin levels were not attributable to the nutritional status.

One important finding of our study is the correlation of low adiponectin levels with the increasing stage of disease, which is in agreement with the currently accepted hypothesis that adiponectin may exert protective actions through its anti-proliferative and anti-angiogenic effects (11, 12). Another possibility is that the low adiponectin levels might result from the increased tumor burden, which is in agreement with the finding that expression and secretion of adiponectin were significantly reduced by TNF-α in a dose- and time-dependent manner *via* its promoter activity (15). Indeed, many tumors, including CRC, produce various inflammatory cytokines and the levels of TNF-α have been shown to be associated with advanced stage of CRC (16). On the other hand, adiponectin has also been shown to inhibit the production of TNF-α in macrophages and its action in endothelial cells, suggesting that low adiponectin levels could potentially lead to carcinogenesis by changing the influence of TNF-α on tumor cell proliferation (11). Thus, the issue of whether adiponectin changes are directly linked to cancer development or whether they simply correlate with metabolic dysfunction is still open to question.

Although the findings obtained in this study do not allow us to draw any conclusion on the mechanisms responsible for the relationship between adiponectin and cancer progression, the association found with tumor stage and, most importantly, the predictive value of low adiponectin levels with respect to disease-free survival suggest that this

Table III. *Multiple regression analysis of the variables associated with low adiponectin levels in non-metastatic colorectal cancer patients.*

Entire model				
Dependent variable	Explanatory variable	Standardized regression co-efficient	Standard error	*p*-value
Adiponectin				
	Male gender	0.422074	0.120303	0.000951
	Age	0.001226	0.005808	0.833664
	Site	0.002214	0.090649	0.980610
	Grade	0.011046	0.096394	0.909219
	Dukes' stage	–0.220367	0.094725	0.024009
	CA199	–0.003509	0.002506	0.167467
	CEA	0.003313	0.002262	0.149162
	CA 72-4	–0.005525	0.016270	0.735562
Forward stepwise method				
	Male gender	0.430824	0.107373	0.000177
	Dukes' stage	–0.222684	0.085555	0.011764

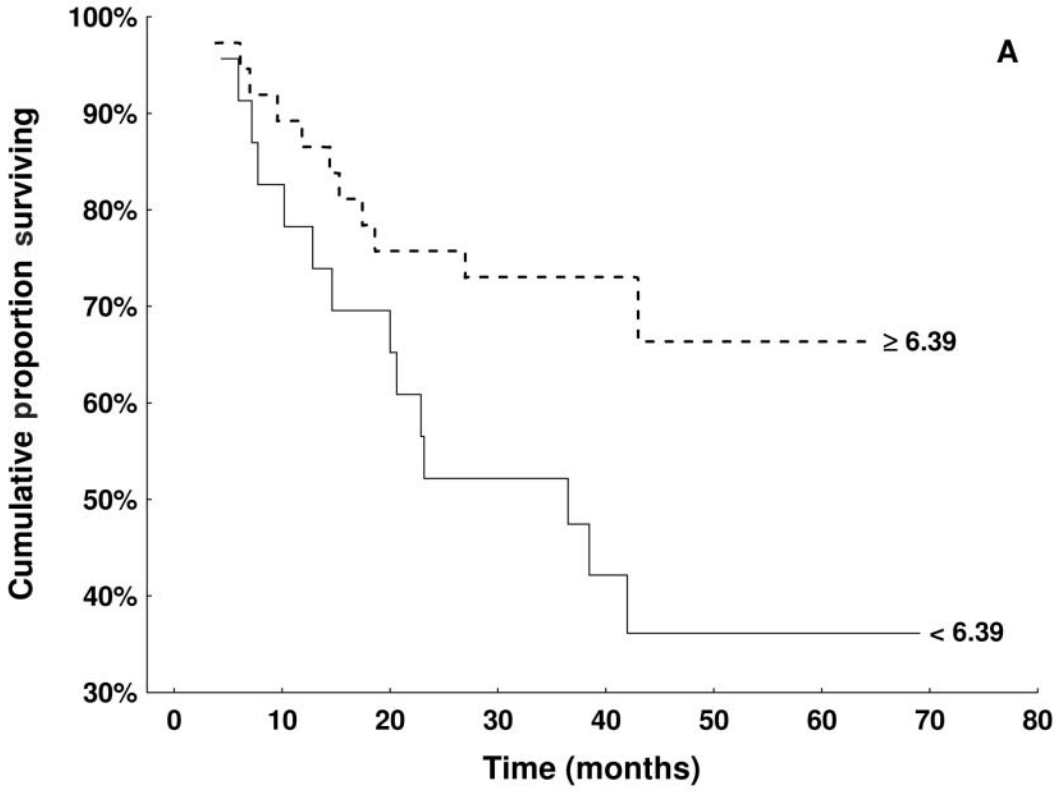
*Categorized according to a cut-off value calculated from the mean value – 2SD of the values observed in the control subjects.

Table IV. *Logistic regression analysis of relapse-free survival in non-metastatic colorectal cancer patients.*

		Recurrence				
Variable	N	Yes	No	OR	95% CI	*p*-value
Gender						
Female	29	12 (41)	17 (59)			
Male	31	13 (42)	18 (58)	2.21	0.47-10.42	0.316
Site						
Colon	15	6 (40)	9 (60)			
Sigma	14	3 (21)	11 (79)			
Rectum	31	16 (52)	15 (48)	1.03	0.43-2.44	0.950
Dukes' stage						
A	7	0 (0)	7 (100)			
B	34	12 (35)	22 (65)			
C	19	13 (68)	6 (32)	15.9	1.98-127.9	< 0.01
Adiponectin						
*Low	22	13 (59)	9 (41)			
High	38	12 (32)	26 (68)	4.66	0.95-22.9	< 0.05
CEA						
Negative	45	16 (36)	29 (64)			
Positive	15	9 (60)	6 (40)	2.01	0.38-10.5	0.408
CA 19-9						
Negative	52	20 (38)	32 (62)			
Positive	8	5 (63)	3 (37)	2.49	0.33-18.8	0.376
CA 72-4						
Negative	51	21 (41)	30 (59)			
Positive	9	4 (44)	5 (56)	0.64	0.10-3.89	0.625

Numbers in parenthesis represent percentages.
*Below (low) and above (high) the mean – 2SD of the control subjects.

adipokine might represent an adjunctive tool in risk prediction for CRC recurrence. It is worth noticing that of the 25 patients who had recurrent disease, 52% had low pre-surgical adiponectin levels compared to approximately 26% of the patients who remained free of disease, thus, making low adiponectin levels a strong predictor of recurrence in the overall population (OR=4.66, $p<0.05$) independently of tumor stage.



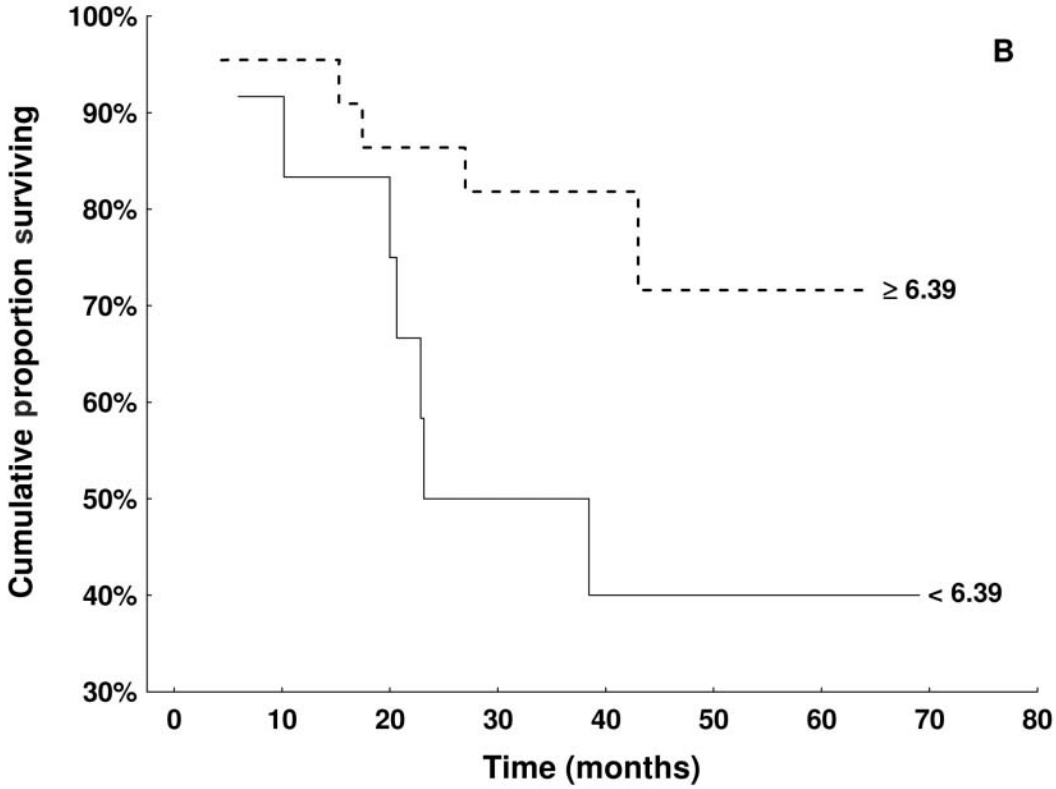


Figure 2. *Kaplan-Meier Analysis of relapse-free survival time of non-metastatic CRC patients. Comparison between patients with low (solid line) and high (dotted line) adiponectin levels (cut-off 6.39 μg/ml, mean −2SD of controls). A: overall population (n=60) (Log-rank statistic=2.11, p=0.035); B: patients with Dukes' stage B disease (n=34) (Log-rank statistic=1.94, p=0.053).*

One limitation of this study was that detailed information on anthropometric measures was not available to the investigators. Despite this limitation, this is the first study, to our knowledge, on adiponectin levels in CRC and supports the hypothesis that this adipokine may have an independent prognostic role in predicting relapsing disease. While this hypothesis requires detailed experimental evaluation before its ultimate significance can be determined; it is hoped that new studies will be undertaken to fully elucidate the mechanisms underlying the adiponectin effects and to better understand its significance in disease progression, as well as its contribution as a prognostic factor for CRC. Nonetheless, our results suggest that the determination of serum adiponectin levels might represent a prognostic parameter in the management of patients with CRC, and may help in the choice of more aggressive treatment and/or more strict follow-up procedures in a subset of patients who are at high risk of recurrence.

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