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ORIGINAL RESEARCH

Role of VEGF, CD105, and CD31 in the Prognosis of Colorectal Cancer Cases

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Abstract
Introduction Colorectal cancer (CRC) incidence is increasing globally. It is ranked as the second most common cancer in women and the third most in men. Angiogenesis plays a significant role in the development and spread of colorectal cancer. Angiogenesis has been proposed as a prognostic marker in a variety of human neoplasms. In this regard, markers of angiogenic endothelial cells are emerging as targets for cancer therapy.
Aim of the Work The aim of this study is to evaluate the prognostic impact of tumor angiogenesis assessed by microvessel density (MVD) counting using CD31 and CD105 along with VEGF immunostaining in colorectal cancer patients.
Methods VEGF, CD31, and CD105 expressions were evaluated using immunohistochemical staining in 50 patients with colorectal cancer. The relationship between their expressions and clinicopathological factors and outcome of patients were analyzed.
Results The VEGF expression (70% of the cases) correlated significantly with larger tumor size, higher grade, and advanced tumor stage ($p = 0.006$, $p < 0.001$, $p < 0.001$), respectively. The mean MVD was 24.2 ± VMD by CD105 (p = 0.10.65 019 for CD105, 19.2 ± 8.41 for CD31, respectively. MVD by CD31 ($p = 0.023$)) and was significant predictive factors for overall survival. Furthermore, the VEGF expression (p = < 0.001) was a significant predictive factor for DFS. There was a statistically significant association between the recurrence rates with both VEGF and CD105 ($p < 0.001$) but not significant with CD31.
Conclusion CRC patients with high VEGF, CD105, and CD31 expression showed poor prognosis. The immunohistochemical markers could be used for stratification of patients into low-risk and high-risk groups.

Keywords VEGF · CD105 · CD31 · Colorectal cancer

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Introduction

The incidence of colorectal cancer (CRC) is increasing globally [18]. It is ranked as the second most common cancer in women (9.2% of all cancers) and the third most in men (10.0% of all cancers) (WHO [40]). According to the National Cancer Institute (NCI), cancer pathology registry 2003–2004; CRC accounts for 4.34% of all cancers in Egypt [26].

Patients with colorectal cancer, except for the advanced stage, undergo curative resection. Stage II patients and stage III patients will take adjuvant chemotherapy after surgical resection [34].

Stage II CRC is a heterogeneous group of cancers with different biology. While the benefit of adjuvant chemotherapy has been demonstrated in stage III disease, it remains controversial for stage II patients [21]. In this regard, additional studies must be focused on developing prognostic biomarkers to identify the subgroup of stage II patients with an increased risk of cancer recurrence after curative surgical resection.

Prognostic factors provide targeted therapy to improve the clinical outcome [34].

Angiogenesis has been proposed as a prognostic marker in a variety of human neoplasms [32].

Angiogenesis plays a significant role in the development and spread of colorectal cancer; the known prognostic factors cannot predict the outcome as tumor size and lymph node involvement. Microvessel density (MVD) counting inside the tumor by using immunohistochemical staining of endothelial cells is suggested to have a prognostic role [38].

The angiogenesis could be identifyied with the pan-endothelial markers CD31 and CD34 and also with CD105 (endoglin).

VEGF acts as a potent tumor angiogenic factor [6]. It stimulates the growth of new blood vessels, which provide tumors with needed oxygen and nutrients (Bergers and Benjamin [4]). Its activity is mainly on the vascular endothelial cells being associated with vascular invasion [17].

CD31 stains both standard and intratumoral blood vessels [27]. CD105 is found in patient serum and expressed mainly on proliferated vascular endothelial cells, so it is preferred in detecting tumor-associated newly formed vessels [9].

Assessing the role of angiogenesis based markers in prognosis could help in determining the appropriate methods of treatment of CRC patients. So, we aimed to evaluate the prognostic impact of tumor angiogenesis assessed by MVD counting using CD31 and CD105 along with VEGF immunostaining in colorectal cancer patients.

Patients and Method

Patients

This study was conducted in the Pathology department and department of surgery in collaboration with the internal medicine department, faculty of medicine, Zagazig University, in the period from January 2014 to December 2016. The study was carried out on 50 patients with colorectal carcinoma (CRC). Cases of CRC were examined by two independent pathologists, classified and graded according to the World Health Organization (WHO) criteria (Hamilton et al. [15]) and standards of the American Joint Committee on Cancer Prognostic (Redston [29]). Clinical, radiological, and pathological data were abstracted from files of the corresponding departments. Clinical follow-up was done every 3 months to all cases, and information concerning follow-up was abstracted from hospital records or patient contact. None of the patients had received chemo or radiotherapy preceding surgery. The Ethics Committee of the Zagazig University Hospital authorized the collection of specimens.

Methods

Immunohistochemical staining was carried out using the streptavidin-biotin immunoperoxidase technique. Sections of 4–5-μm thickness, cut from formalin-fixed paraffin embedded blocks, were deparaffinized in xylene and rehydrated in graded alcohol. Antigen retrieval was performed using microwave treatment in 10 mmol/l citrate buffer, pH 6. These sections were washed three times with cold 0.01 mol/l PBS. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min. The used primary antibodies/monoclonal mouse antibodies anti-VEGF (dilution 1:100; clone: VG; anti CD31 (clone: JC/70A); 0.1 ml pre-diluted rabbit polyclonal Anti Cd105 (ab27422) as well as the detection kit (UltraVision Detection System Anti-polyvalent, HRP/DAB, Ready-To-Use) were purchased from the Thermo Scientific Lab Vision, USA. Incubation with a secondary antibody and product visualization were performed with diaminobenzidine substrate as the chromogen. The slides were finally counterstained with Mayer's hematoxylin. Positive and negative controls were included in all runs.

Evaluation of Immunostaining

The criterion for a positive immune reaction for VEGF is a dark brown cytoplasmic precipitate. The staining intensity was scored as following: negative, (weak) = less than 10%, (moderate) = 11 to 50%, and (strong) = more than 50% tumor cells stained positive. [2, 20].

Microvessel Density Evaluation by CD31, CD105

Microvessel density was assessed by immunostaining for CD31 and CD105 according to Weidner [39]. A single brown staining endothelial cell or small clusters of brown staining endothelial cells, with or without lumen, can be considered as individual vessels. Slides were examined at low power magnification to find the areas of greater concentration of stained vessels "hot spots"; three sectors which showed the highest concentration of microvessels were selected. Each zone was examined with the high-power field to identify the maximum number of microvessels. The highest value obtained among the three fields was reported as MVD [19].

Statistical Analysis

All statistics were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium) using Pearson's chi-squared test or Fisher's exact test. Overall survival (OS) was calculated as the time from diagnosis to death. disease-free survival (RFS) was calculated as the time from start of treatment to date of relapse. The stratification of OS and disease-free survival (DFS) was done according to markers using the method of Kaplan-Meier plot and compared using two-sided exact log-rank test. A significance level of $p < 0.05$ was used in all tests.

Results

Patient Data

Fifty patients with CRC, 21 males (42%) and 29 females (58%), were enrolled in this study, with age ranged from 32 to 66 years (median age 50 years). The demographic data of our patients were shown in Table 1.

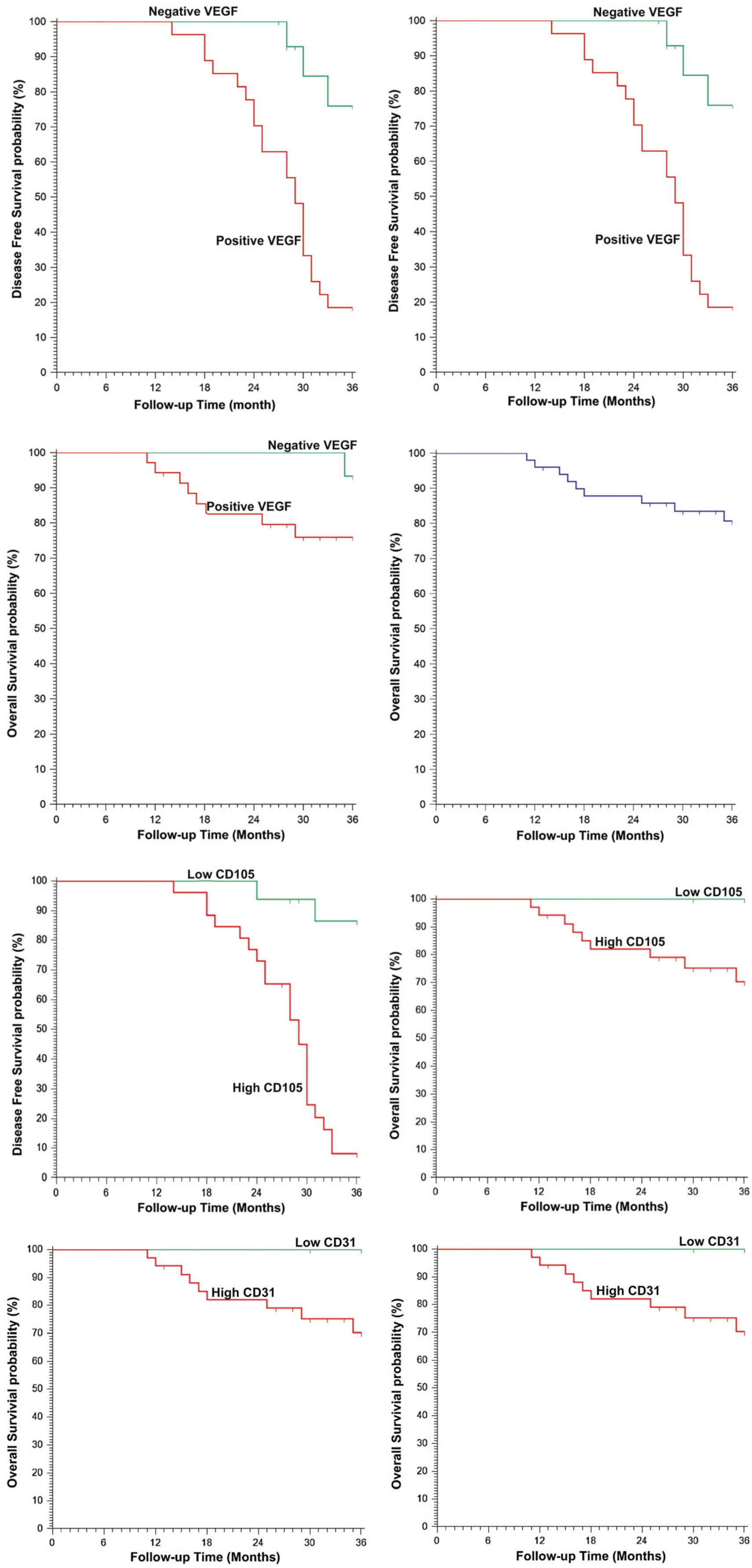
Immunohistochemical Results

A VEGF positive expression was detected in 35 out of 50 (70%) of CRC cases. High VEGF expression was correlated with larger tumor size ($p = 0.006$). The association of VEGF staining with higher grade, high incidence of lymph node metastases, and the advanced stage was statistically significant ($p < 0.001$) (Figs. 1 and 2, Table 2). Furthermore, VEGF was correlated to MVD obtained by both CD105 and CD31immunostaining ($p < 0.001$ and 0.035), respectively (Table 3, Figs. 1 and 2).

MVD of all cases stained by CD31 or CD105 was obtained then the mean was calculated .the mean for each marker is considered the cutoff point. Values above it is seen as high

Table 1 Clinicopathological features, immunohistochemical markers, and outcome of 50 patients with CRC

		All (N = 50)				All (N = 50)	
		No.	%			No.	%
Age (years)	Mean ± SD	50.60	±8.16	T	T2	12	24%
	Median (range)	50	(32–66)		T3	21	42%
	≤ 50 years	28	56%		T4	17	34%
	> 50 years	22	44%				
Sex	Male	21	42%	N	N0	29	58%
	Female	29	58%		N1	10	20%
					N2	11	22%
Site	Right colon	15	30%	M	M0	42	84%
	Transverse colon	6	12%		M1	8	16%
	Left colon	19	38%				
	Rectosigmoid	10	20%				
Gross pattern	Ulcerative	14	28%	AJCC stage	Stage I	9	18%
	Fungating	11	22%		Stage II	20	40%
	Annular	25	50%		Stage III	13	26%
					Stage IV	8	16%
Size (cm)	Mean ± SD	5.10	±1.59	VEGF	Negative	15	30%
	Median (range)	5	(2–10)		Positive	35	70%
	< 5 cm	19	38%				
	5–10 cm	31	62%				
Histopathology	Adenocarcinoma	42	84%	CD105 MVD	Low ≤ 25	16	32%
	Mucinous	8	16%		High > 25	34	68%
Grade	Grade I	17	34%	CD31 MVD	Low ≤ 20	22	44%
	Grade II	12	24%		High > 20	28	56%
	Grade III	21	42%				
LVI	Absent	23	46%	Follow-up (months)	Mean ± SD	31.22	± 7.57
	Present	27	54%		Median (range)	36	(11–36)
Lymph nodes	Node negative	29	58%	Relapse	Absent	17	40.5%
	Node positive	21	42%		Present	25	59.5%
Distant metastasis	Absent	42	84%	Death	Alive	41	82%
	Present	8	16%		Died	9	18%


Negative VEGF
Positive VEGF
Disease Free Survivial probability (%)
Follow-up Time (month)
Negative VEGF
Positive VEGF
Disease Free Survivial probability (%)
Follow-up Time (Months)
Negative VEGF
Positive VEGF
Overall Survivial probability (%)
Follow-up Time (Months)
Overall Survivial probability (%)
Follow-up Time (Months)
Low CD105
High CD105
Disease Free Survivial probability (%)
Follow-up Time (Months)
Low CD105
High CD105
Overall Survivial probability (%)
Follow-up Time (Months)
Low CD31
High CD31
Overall Survivial probability (%)
Follow-up Time (Months)
Low CD31
High CD31
Overall Survivial probability (%)
Follow-up Time (Months)

◀ **Fig. 1** Relation between clinicopathological features and immunohistochemical staining for VEGF, CD105, and CD31 in 50 patients with colorectal carcinoma

MVD while values less than the mean are considered low MVD, according to Abdel Dayem et al. [1].

In the current study, CD105 was used to highlight blood vessels which were variable as regards their size and morphology; they were unevenly distributed in the tumor. MVD of 50 CRC patients was ranged from 10 to 35 with mean 24.2 ± 10.65. According to the value of MVD, the examined group was divided into two groups: low and high; the cutoff point to distinguish low MVD from high MVD was 25.

High CD105 MVD was detected in 34 out of 50 (68%) of the CRC cases. It was correlated with larger tumor size ($p = 0.014$) and mucinous histologic type ($p = 0.043$). There was a significant association between CD105 and higher tumor grade, high incidence of lymph node metastases, and advanced stage ($p < 0.001$) for all. CD105 MVD was correlated to VEGF and CD31 immunostaining ($p < 0.001$ and $p = 0.016$), respectively (Tables 2 and 3, Figs. 1 and 2).

MVD was studied by using CD31 which ranged from 10 to 30 with mean19.2 ± 8.41. Examined cases were classified according to the MVD mean value into two groups, the low MVD group which is less than 20 and the high MVD group more than 20. High MVD was detected in 28 out of 50 (56%) of the CRC cases. CD31 was correlated with larger tumor size, higher grade, and higher tumor stage ($p = 0.033$, $p = 0.028$, and $p = 0.004$), respectively. No significant association was detected with lymph node metastasis. CD31 showed a significant correlation to both VEGF and MVD obtained by CD105 immunostaining ($p = 0.035$ and $p = 0.016$) respectively (Tables 2 and 3, Figs. 1 and 2).

Recurrence and Survival Analysis

In the examined cases, relapse was detected in 25 and absent in 17 cases out of 42 cases exposed to analysis. It was clear

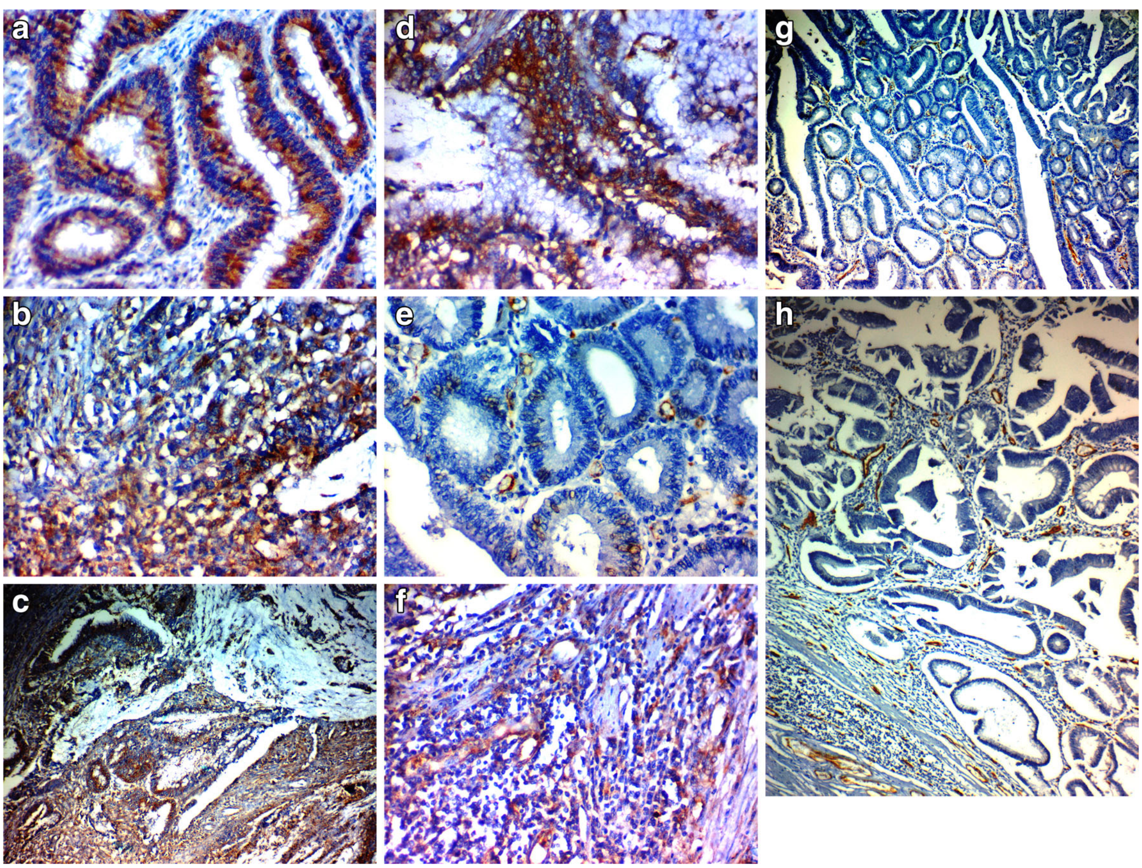


Fig. 2 **a** Immunohistochemical staining of VEGF in low-grade colon adenocarcinoma ×400 magnification. **b** Immunohistochemical staining of VEGF in high-grade colon adenocarcinoma ×400. **c** Immunohistochemical staining of VEGF in mucinous colon carcinoma ×200. **d** Immunohistochemical staining of VEGF in mucinous colon carcinoma ×400. **e** Immunohistochemical staining of cd105 in low-grade colon adenocarcinoma ×200 magnification. **f** Immunohistochemical staining of cd105 in high-grade colon adenocarcinoma ×400 magnification. **g** Immunohistochemical staining of cd31 in low-grade colon adenocarcinoma ×100 magnification. **h** Immunohistochemical staining of cd31 in moderate grade colon adenocarcinoma ×200 magnification

Table 2 Relation between clinicopathological features and immunohistochemical staining for VEGF, CD105, and CD31 in 50 patients with colorectal carcinoma

			VEGF			CD105MVD			CD31 MVD		
		No	*N*	*p*	*p*	Low	High	*p*	Low	High	*p*
N		(50)	(15)	(35)		(16)	34		(22)	(28)	
Age (years)	≤ 50	28	8	20	0.804[a]	9	19	0.981[a]	9	19	0.057[a]
	> 50	22	7	15		7	15		13	9	
Sex	Male	21	8	13	0.288[a]	7	14	0.863[a]	10	11	0.661[a]
	Female	29	7	22		9	20		12	17	
Site	Right colon	15	0	15	0.021[a]	3	12	0.419[a]	4	11	0.172[a]
	Transverse colon	6	2	4		1	5		2	4	
	Left colon	19	8	11		8	11		12	7	
	Rectosigmoid	10	5	5		4	6		4	6	
Gross pattern	Ulcerative	14	6	8	0.456[a]	5	9	0.904[a]	8	6	0.328[a]
	Fungating	11	3	8		3	8		3	8	
	Annular	25	6	19		8	17		11	14	
Size	< 5 cm	19	10	9	0.006[a]	10	9	0.014[a]	12	7	0.033[a]
	5–10 cm	31	5	26		6	25		10	21	
Histopathological type	Adenocarcinoma	42	13	29	1.000[a]	16	26	0.043[a]	21	21	0.064[a]
	Mucinous	8	2	6		0	8		1	7	
Grade	Grade I	17	14	3	< 0.001[b]	13	4	< 0.001[b]	11	6	0.028[b]
	Grade II	12	1	11		2	10		5	7	
	Grade III	21	0	21		1	20		6	15	
LVI	Absent	23	15	8	< 0.001[a]	16	7	< 0.001[a]	12	11	0.283[a]
	Present	27	0	27		0	27		10	17	
Lymph nodes	Negative	29	14	15	0.001[a]	15	14	< 0.001[a]	16	13	0.061[a]
	Positive	21	1	20		1	20		6	15	
Distant metastasis	Absent	42	15	27	0.086[a]	16	26	0.043[a]	22	20	0.006[a]
	Present	8	0	8		0	8		0	8	
T	T2	12	8	4	< 0.001[b]	7	5	0.003[b]	7	5	0.117[b]
	T3	21	7	14		8	13		10	11	
	T4	17	0	17		1	16		5	12	
N	N0	29	13	16	0.014[b]	14	15	0.003[b]	14	15	0.160[b]
	N1	10	1	9		2	8		6	4	
	N2	11	1	10		0	11		2	9	
M	M0	42	15	27	0.086[a]	16	26	0.043[a]	22	20	0.006[a]
	M1	8	0	8		0	8		0	8	
AJCC stage	Stage I	9	7	2	< 0.001[b]	6	3	< 0.001[b]	7	2	0.004[b]
	Stage II	20	7	13		9	11		9	11	
	Stage III	13	1	12		1	12		6	7	
	Stage IV	8	0	8		0	8		0	8	

Categorical variables were expressed as number (percentage); continuous variables were expressed as mean ± SD and median (range); independent samples Student's test; Mann-Whitney *U* test; $p < 0.05$ is significant

[a] Chi-squared test

[b] Chi-squared test for trend

that relapsing disease significantly correlated with large tumor size, higher grade, higher stage, and lymph node status ($p = 0.006$, 0.005, 0.001, and 0.004), respectively (Table 5).

A negative expression of VEGF and low MVD obtained by CD105 were significantly associated with low recurrence and vice versa; their high expression levels related to increased relapse incidence < 0.001 for both markers. On the contrary, there was no correlation found to CD31MVD with disease recurrence (Table 4, Fig. 1).

High expression levels of VEGF were also associated with shorter duration of disease-free survival ($p < 0.001$); CD105 high MVD values are related to both shortened DFS, overall

Table 3 Relation between immunohistochemical staining for VEGF, CD105, and CD31 in 50 patients with colorectal carcinoma

			VEGF		p value	CD105 MVD		p value	CD31 MVD		p value
			Negative (N = 15)	Positive (N = 35)		Low (N = 16)	High (N = 34)		Low (N = 22)	High (N = 28)	
VEGF	Negative	15				11	4	< 0.001[a]	10	5	0.035[a]
	Positive	35				5	30		12	23	
CD105MVD	Low	16	11	5	< 0.001[a]				11	5	0.016[a]
	High	34	4	30					11	23	
CD31MVD	Low	22	10	12	0.035[a]	11	11	0.016[a]			
	High	28	5	23		5	23				

Categorical variables were expressed as number (percentage),; $p < 0.05$ is significant

[a] Chi-squared test

survival OS, and also associated with high mortality rates (p = <0.001, 0.019, 0.043), respectively. High values of CD31 MVD found to be significantly related to the decreased patient OS ($p = 0.023$) (Table 4, Fig. 1).

Discussion

There are controversies regarding the results of the published literature relating to the angiogenesis in CRC. One of the reasons is the large panel of antibodies and also the different methods utilized for quantification [12].

Angiogenesis is a classical hallmark of cancer. The antiangiogenic therapy can target the endothelial cells directly by suppressing the production and or the action of pre angiogenic peptides or indirectly by augmenting the release of antiangiogenic factors within the tumor [16, 28]. In this regard, markers of angiogenic endothelial cells are emerging as targets for cancer therapy [24].

The current study has been evaluated the prognostic impact of tumor angiogenesis assessed by vascular endothelial growth factor (VEGF), CD31, and CD105 in colorectal cancer patients (Tables 5 and 6).

VEGF is a key mediator of angiogenesis in cancer and considers an adverse prognostic marker in CRC [8, 33].

The present study assessed that the VEGF positive expression in 70% of the cases which is nearly to Saad et al.'s [31] study in which VEGF was demonstrated in 67% of the cases. The VEGF positive expression was significantly associated with large tumor size with $p = 0.006$, also associated with higher grade, lymphovascular invasion, high incidence of positive lymph nodes, and advanced stage of cancer with $p < 0.01$, which was close to Ferroni et al. [13] who disclosed that positive VEGF expressions were significantly associated with high grade and advanced stage tumors ($p = 0.04$) and ($p < 0.05$), respectively. Dassoulas et al. [10] approved no significant correlation of the VEGF expression with clinicopathological parameters which were not by our results.

Chung et al. [8] and Mohamed et al. (2016) also confirmed a VEGF association with high-grade cases. Similar results were found by Saad et al. [31] declaring a significant association between VEGF expression and tumor grade, LN involvement, and lymphovascular invasion ($p < 0.05$).

Our results identified a strong direct relationship of VEGF with relapsing CRC ($p < 0.01$) and with shorter disease-free survival ($p < 0.01$); no correlation was found between VEGF and patient overall survival, which was close to Khorana et al. [22] and Chung et al. [8] who did not show association with patient overall survival and was not in accordance to Bendardaf et al.[3] who suggested that the VEGF expression was associated with less favorable long-term survival as compared with VEGF-negative tumors. This contradiction may be due to changes in some examined cases.

The present study found that high CD31 MVD was significantly associated with larger tumor size ($p = 0.003$), higher grade ($p = 0.028$), and advanced stage of cancer $p = 0.004$; our results were similar to that of Tien et al. [36] who approved that Cd31 was significantly related to tumor stage = 0.002. In Cammarota et al.'s [5] study, Cd31 was correlated with an increasing grade of malignancy which is similar to our results. Saad et al. [31] assessed a correlation of CD31 MVD to tumor size, but they found a correlation with angiolymphatic invasion and lymph node metastases which were in contradiction to the results of the present study.

In the present study, the association also was found between CD31 and short overall survival ($p = 0.023$) but no association was found to disease recurrence. Gulubova and Vlaykova [14] reviewed a positive correlation between CD31 and poor survival which is in agreement with the current study.

The CD105 seems to be the best marker for a study of neoangiogenesis in CRC. CD105 can mark the activated endothelium of preexistent mature vessels. The radiotherapy destroys the neoformed but also the pre-existent vessels [20].

Table 4 Relation between immunohistochemical staining for VEGF, CD105, and CD31 and outcome in 50 patients with colorectal carcinoma

			VEGF		*p*	CD105 MVD		*p*	CD31 MVD		*p*
		N	Negative (*N* = 15)	Positive (*N* = 35)		Low < 25 (*N* = 16)	High > 25 (*N* = 34)		Low < 20 (*N* = 22)	High > 20 (*N* = 28)	
Relapse	Absent	17	12	5	< 0.001	14	3	< 0.001	12	5	0.051
	Present	25	3	22		2	23		10	15	
DFS	Mean (months) (95% CI)	30.20 months (28.38–32.03)	34.67 months (33.27–36.07)	27.89 months (25.61–30.17)	< 0.001	34.89 months (33.35–36.43)	27.38 months (25.24–29.52)	< 0.001	31.19 months (28.66–33.71)	29.15 months (26.59–31.71)	0.112
	One-year DFS	100%	100%	100%		100%	100%		100%	100%	
	Two-year DFS	81%	100%	70.4%		93.8%	73.1%		81.8%	80%	
	Three-year DFS	37.2%	76%	18.5%		86.5%	8.2%		52.2%	21.4%	
Death	Alive	41	14	27	0.247	16	25	0.043	21	20	0.060
	Died	9	1	8		0	9		1	8	
OS	Mean (months) (95% CI)	33.01 months (31.04–34.99)	35.93 months (35.81–36.06)	31.72 months (28.99–34.46)	0.125	36 months	31.54 months (28.75–34.34)	0.019	35.50 months (34.54–36.46)	31 months (27.71–34.29)	0.023
	One-year OS	96%	100%	94.3%		100%	94.1%		100%	92.9%	
	Two-year OS	87.8%	100%	82.5%		100%	82%		100%	78%	
	Three-year OS	80.7%	93.3%	75.9%		100%	70.2%		95.5%	68.4%	

Table 5 Relation between clinicopathological features and relapse in 42 patients with colorectal carcinoma

Characteristics		*All*	Relapse		*p*
		(*N* = 42)	Absent (*N* = 17)	Present (*N* = 25)	
Age	≤ 50 years	24	9	15	0.650
	> 50 years	18	8	10	
Sex	Male	16	8	8	0.324
	Female	26	9	17	
Site	Right colon	11	1	10	0.068
	Left colon	6	2	4	
	Transverse colon	18	10	8	
	Rectosigmoid	7	4	3	
Gross pattern	Ulcerative	11	6	5	0.536
	Fungating	9	3	6	
	Annular	22	8	14	
Size	< 5 cm	19	12	7	0.006
	5–10 cm	23	5	18	
Histopathology	Adenocarcinoma	39	17	22	0.260
	Mucinous	3	0	3	
Grade	Grade I	17	12	5	0.005
	Grade II	11	2	9	
	Grade III	14	3	11	
Lymphovascular invasion	Absent	23	16	7	< 0.001
	Present	19	1	18	
Lymph nodes	Node negative	29	16	13	0.004
	Node positive	13	1	12	
T	T2	12	9	3	0.001
	T3	18	7	11	
	T4	12	1	11	
N	N0	29	15	14	0.016
	N1	7	2	5	
	N2	6	0	6	
AJCC stage	Stage I	9	8	1	< 0.001
	Stage II	20	8	12	
	Stage III	13	1	12	

The present study assessed that high CD105 MVD was significantly associated with larger tumor size (p = 0.014), mucinous histologic type = 0.043, higher grade, high incidence of lymph node positivity, and advanced stage of cancer ($p < 0.01$), and these results were from Deliu et al.'s [11] study. Also, these results were near to that of Saad et al. [31] who disclosed that the endoglin was correlated with high-grade lesions, angiolymphatic invasion, lymph node positivity, and metastatic cases. However, no significant correlation of endoglin expression with clinicopathological parameters was observed in Dassoulas et al.'s [10] study.

CD105 are correlated with higher incidence of recurrence, short duration of DFS ($p < 0.01$), and also correlated with poor overall survival and increased mortality rates (p = 0.019, p = 0.043).

Li et al. [23] disclosed that determining MVD by using CD105 was strongly associated with a poor prognosis and could be considered as an independent prognostic parameter for survival in colorectal cancer patients which was in agreement with our results. Chen et al. [7] have reported that the CRC with higher MVD is more likely to recur or metastasize after radical resection. However, Uribarrena et al. [37] observed no relation between MVD and tumor recurrence and death.

Takahashi et al. [35] suggested that serum endoglin may be a useful marker for monitoring early signs of metastasis and cancer relapse.

In our study, we observed that tumor blood vessel counting obtained by CD105 MVD was higher than CD31MVD, with a strong association between both markers. This result was in agreement with Saad et al. [31] who demonstrates that the CD105 is the best marker to identify proliferating endothelium.

While in some studies, the MVD determined with CD31 was higher than that for CD105, explaining that by, CD31 also

Table 6 Relation between clinicopathological features and mortality in 50 patients with colorectal carcinoma

		(*N* = 50)	Alive (*N* = 41)	Died (*N* = 9)	
Age (years)	Mean ± SD	50.60 ± 8.16	49.68 ± 7.60	54.77 ± 9.74	0.090
	Median (range)	50 (32–66)	49 (32–65)	55 (40–66)	
	≤ 50 years	28	24	4	0.481
	> 50 years	22	17	5	
Sex	Male	21	17	4	1.000
	Female	29	24	5	
Site	Right colon	15	9	6	0.039
	Transverse colon	6	6	0	
	Left colon	19	18	1	
	Recto sigmoid	10	8	2	
Gross pattern	Ulcerative	14	14	3	0.506
	Fungating	11	11	3	
	Annular	25	25	3	
Size (cm)	Mean ± SD	5.10 ± 1.59	4.78 ± 1.25	6.55 ± 2.18	0.043
	Median (range)	5 (2–10)	5 (2–8)	6 (3–10)	
	< 5 cm	19	18	1	0.127
	5–10 cm	31	23	8	
Histopathology	Adenocarcinoma	42	38	4	0.003
	Mucinous	8	3	5	
Grade	Grade I	17	16	1	0.008§
	Grade II	12	12	0	
	Grade III	21	13	8	
Lymphovascular invasion	Absent	23	22	1	0.028
	Present	27	19	8	
Lymph nodes	Node negative	29	29	0	< 0.001
	Node positive	21	12	9	
Distant metastasis	Absent	42	39	3	< 0.001
	Present	8	2	6	
T	T2	12	11	1	0.048§
	T3	21	19	2	
	T4	17	11	6	
N	N0	29	28	1	0.001§
	N1	10	7	3	
	N2	11	6	5	
M	M0	42	39	3	<0.001
	M1	8	2	6	
AJCC stage	Stage I	9	9	0	<0.001§
	Stage II	20	20	0	
	Stage III	13	10	3	
	Stage IV	8	2	6	
Relapse	Absent	17	17	0	0.260
	Present	25	22	3	

assesses the pre-existent mature vessels and neoformation vessels [11].

This discrepancy may be due to the counting procedures for assessment of MVD, which are not standardized; the use of different antibodies for immunohistochemistry; the various numbers of microscopic fields; and the various magnifications used.

CD105 is preferentially expressed in vessels undergoing neoangiogenesis, while CD31 stained all the blood vessels with inconsistent staining reliability between laboratories. Therefore, endoglin staining should reduce false-positive staining of blood vessels when compared with other commonly used pan-endothelial markers [30].

Being correlated with poor patient survival, either OS or DFS or both, MVD counting by using CD31 and CD105 together with VEGF immunostaining may help in prognosis detection and stratification of patients into low- and high-risk groups, which determine the need for postoperative adjuvant chemotherapy for patients in the high-risk group.

Conclusion

Colorectal cancer patients with higher expression of VEGF, high values of CD105, and CD31 MVD showed high recurrence rates and short survival; these immunohistochemical markers could be used for stratification of patients into low-risk and high-risk groups which aid in the identification of the appropriate treatment modalities for different cases.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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