ORIGINAL ARTICLE
(CC BY-SA)
UDC: 616.359-006-037
https://doi.org/10.2298/VSP180120071S

Vascular endothelial growth factor as a potential prognostic factor for T3N0 rectal cancer

Vaskularni endotelni faktor rasta kao potencijalni prognostički faktor kod T3N0 stadijuma karcinoma rektuma

Aleksandar Sekulić*, Goran Barišić*†, Duško Dundjerović‡, Svetislav Tatić‡, Zoran Krivokapić*†

Clinical Centre of Serbia, First Surgical Clinic, *Clinic for Digestive Surgery, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, ‡Institute of Pathology, Belgrade, Serbia

Abstract

Background/Aim. Rectal cancer still presents a major health problem. Although a surgery is the mainstay of the rectal cancer treatment, there is now widespread agreement that combined modality therapy is often indicated. Around 20% of T3N0 rectal cancer patients develop distant or local relapse of the disease. There is a need for prognostic biomarkers that could help us determine the subgroup of patients with a high risk for recurrence. The aim of this study was to determine the prognostic potential of vascular endothelial growth factor (VEGF) in patients with T3N0 rectal carcinoma. **Methods.** This retrospective study included 163 selected T3N0 rectal cancer patients, operated on the Department for Colorectal Surgery of the Clinic for Digestive Surger (First Surgical Clinic), Clinical Centre of Serbia, Belgrade. VEGF expression was immunohistochemically assessed. Oncological outcome was analyzed using data from prospectively designed data base. Parameters of interest were: distant metastases, the disease free and overall survival. Survival and time to recurrence were evaluated using Kaplan Meier`s method and the factors were compared with the long-rank test. **Results.** There were 102 men and 61 women. The median age was 62 years (age range, 31–88 years). Median follow-up interval was 81 months (range, 4–177 months). During the follow-up period 6 patients developed local recurrence, in 31 patients distant metastases occurred. Three factors were found to be associated with distant metastases: VEGF expression, mucinous adenocarcinoma and tumor differentiation ($p < 0.05$). In patients with positive VEGF expression, the disease free survival and overall survival were significantly worse than in negative ones (65% and 59%, respectively) (log-rank test, $p < 0.05$). **Conclusion.** High VEGF expression in T3N0 rectal carcinomas together with some standard histopathological tumor features can give us enough information to identify subgroup of patients with high risk for recurrence and poorer prognosis.

Key words:
biomarkers; prognosis; rectal neoplasms; recurrence; vascular endothelial growth factor.

Apstrakt

Uvod/Cilj. Karcinom rektuma još uvek predstavlja veliki zdravstveni problem. Iako je hirurško lečenje primarno, široko je prihvaćena činjenica da je kombinovana terapija često indikovana u tretmanu ove bolesti. Lokalni ili distalni recidiv javlja se kod oko 20% bolesnika sa karcinomom rektuma T3NO stadijuma. Danas postoji potreba za prognostičkim biomarkerima uz čiju pomoć se mogu predvideti bolesnici sa visokim rizikom od recidiva bolesti. Cilj ove studije bio je da se ispita prognostički potencijal vaskularnog endotelnog faktora rasta (VEGF) kod bolesnika sa T3N0 staadijumom karcinoma rektuma. **Metode.** Retrospektivnom studijom bila su obuhvaćena 163 bolesnika sa T3N0 stadijumom karcinomom rektuma, operisana na III Odeljenju Klinike za digestivnu hirurgiju (Prva hirurška klinika), Kliničkog centra Srbije u Beogradu. Imunohistohemijski je ispitivana ekspresija VEGF. Podaci su prikupljani u prospektivno dizajniranoj bazi podataka. Kao parametri od interesa definisani su pojava udaljenih metastaza i preživljavanje. Preživljavanje i vreme do recidiva bolesti procenjivano je na osnovu Kaplan-Meier-ove metode i *log-rank* testa. **Reziltati.** U studiju su bila uključena 102 muškarca i 61 žena. Prosečna starost ispitanika bila je 62 godine (31–88 godina), a postoperativno praćenje iznosilo je u proseku 81 mesec (4–177 meseci). Kod šest bolesnika je dijagnostiko-

Correspondence to: Aleksandar Sekulić, Clinical Centre of Serbia, First Surgical Clinic, Clinic for Digestive Surgery, Koste Todorovica 6, 11000 Belgrade, Serbia. E-mail: sekulic038@yahoo.com

van lokalni, a kod 31 udaljeni recidiv bolesti. Tri faktora su pokazala značajnu povezanost sa udaljenim metastazama: ekspresija VEGF, mucinozni adenokarcinomi i diferencijacija tumora. Kod bolesnika sa pozitivnom ekspresijom VEGF preživljavanje je bilo lošije u odnosu na bolesnike sa negativnom ekspresijom VEGF (65% i 59%, redom; *log-rank* test $p < 0,05$). **Zaključak.** Povišena ekspresija VEGF kod T3N0 stadijuma karcinoma rektuma, zajedno sa standardnim histopatološkim karakteristikama tumora, može dati dovoljno informacija za definisanje bolesnika sa visokim rizikom od pojave recidiva bolesti i lošijom prognozom.

Ključne reči:
biomarkeri; prognoza; rektum, neoplazme; recidiv; faktori rasta endotela krvnih sudova.

Introduction

Colorectal cancer (CRC) is the major health problem of both developed and some developing countries. The incidence of rectal cancer (RC) in the European Union is ~ 125,000 per year, i.e. ~35% of the total CRC incidence [1].

Biological behavior of RC is in a way different from the colon tumors and its treatment modalities are specific. Besides the need for individualized and meticulous preoperative staging there is sometimes a problem in choosing the optimal mode of treatment. Surgery is still the mainstay of treatment, but neoadjuvant therapy proved to be effective in cases of locally advanced RC [2]. Current problem presents a group of RC patients in T3N0M0 stage. These patients may not benefit from aggressive neoadjuvant and adjuvant approach [3]. Nevertheless, we still have around 20% of patients in this group who develop distant or local relapse of the disease [4, 5]. There is a need for predictive and prognostic markers that could help us determine the subgroup of patients with high risk of relapse [6–8].

Among others, vascular endothelial growth factor (VEGF) has a significant role in angiogenesis, tumor proliferation and metastatic potential. As such it could be used as valuable prognostic tool [9].

The aim of this study was to determine the prognostic potential of VEGF in patients with T3N0 RC in the absence of neoadjuvant treatment.

Methods

This retrospective analysis included patients curatively operated for RC between January 2003 and December 2013. All patients were operated by the same surgical team, on the Department for Colorectal Surgery of the Clinic for Digestive Surgery (First Surgical Clinic), Clinical Centre of Serbia. The patient selection criteria were as follows: without any preoperative therapy; histopathologically confirmed T3N0 rectal adenocarcinoma; no evidence of distant metastases; R0 resections; available to provide follow-up information at least once. Patients deceased within 30 days from operation were excluded from the study. After careful reviewing medical and pathologic records, 163 consecutive patients with T3N0M0 RC were selected. Principles of the standard total mesorectal excision (TME) surgery were uniformly applied. RC was defined as adenocarcinoma located within 15 cm from the anal verge. TME was performed for most patients with mid and distal RC. For upper third RCs partial mesorectal excision was performed with minimal distal clearance of 5 cm. In cases where the restorative prosedure was not possible (cases with external anal sphincter involvement, voluminous tumors with intraoperative perforation, etc.) abdominoperineal excision (APR) or Hartmann's procedure was performed. Pathologic stage and pathologic grade were classified according to the 6th edition of the Union for International Cancer Control (UICC) classification. Oncological outcome of selected patients was analyzed using data from prospectively designed data base. Parameters of interest were: distant metastases, disease free (DFS) and overall survival (OS). Local recurrence (LR) was defined as any histological, morphological or clinical evidence of recurrence of RC within the pelvis, either alone or in association with distant metastases. Distant metastases were defined as the disease recurrence detected in organs excluding the pelvis.

OS was defined as the time from the date of surgery to the date of death or the date of last follow-up of patient who were still alive. DFS was defined as the time from the date of surgery to the date of the diagnosed recurrence. Patients who died without evidence of LR or distant recurrence were censored at the date of death, and patients alive without evidence of LR or distant recurrence were censored at the date of last follow-up.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks from selected patients were used for tissue microarray (TMA) construction. The immunohistochemical detection of VEGF was performed on fresh 3 μm paraffin sections from the TMA block. Slides with 3-μm-thick sections from TMA tissue blocks were dried in a 60°C oven for one hour. The sections were placed in a Bond Max Automated Immunohistochemistry Vision Biosystem (Leica Microsystems GmbH, Germany) according to the following protocol. First, tissues were deparaffinized and pretreated with the Epitope Retrieval Solution 2 at 100°C for 20 min. After washing steps, peroxidase blocking was carried out for 5 min using the Bond Polymer Refine Detection Kit DC9800 (Leica Microsystems GmbH). Slides were again washed and then incubated with the primary antibodies (VEGF, Clone VG1, DAKO, Cat. No M7273, dilution 1 : 50) for 15 min. Subsequently, tissues were first incubated with Post Primary Reagent for 8 min and then with Polymer for 8 min. After washing, sections were developed with DAB-chromogen for 10 min, and counterstained with hematoxylin for 8 min. Omission of the primary antibody was used as a negative control.

Immunohistochemical evaluation

Within tumor cells immunoreactive VEGF protein was detected primarly in the cytoplasm. The evaluation of staining of all TMAs were scored semi-quantitatively by two experienced pathologist blinded to the clinical data. The percentage of positive cells were assessed as follows: 0 – 0% of positive cells; 1 – < 5% of positive cells; 2 – 5%–50% of positive cells; and 3 – > 50% of positive cells. The intensity of staining was scored as: 0 – negative; 1 –weak; 2 – intermediate; and 3 – strong. The final score for the immunoreactions was defined as the sum of both parameters, and grouped as: 0 – negative; 1– weak; 2 – moderate, and 3 – strong. For statistical purposes, only the moderate and strong immunoreactions were considered as positive ones [10].

Statistical analysis

Statistical data analysis was performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Univariate Cox regression model was applied to identify factors affecting distant metastases. A multivariate analysis using the Cox proportional hazards model was performed to investigate the independence of the risk factors identified as significant in the univariate analysis.

Survival was analyzed using Kaplan Meier`s test and the factors were compared with the *log-rank* test. All *p*-values less than 0.05 were considered statistically significant.

Results

Clinical and pathological characteristics of the patients are presented in Table 1. There were 102 (62.6%) men and 61 (37.4) women. Their median age was 62 years (range, 31–88 years). The average number of lymph nodes examined was 22 (range, 4–65). The location of the tumor was in average 9.4 ± 4.2 cm measured from the anal verge. Tumor size (measured as the largest tumor diameter) was 5.7 cm (range, 2–16 cm). Median follow-up interval was 81 months (range, 4–177 months). During the follow-up period 6 patients developed LR, in 31 patients we discovered distant metastases. The 5-year LR and distant metastases rate were 4% and 20%, respectively.

All potential risk factors for distant metastases, including clinicopathologic features and biomarker (VEGF) were evaluated by univariate Cox regression model. Among ten potential prognostic factors only the histological subtype of the tumor, tumor grade, lymphovascular invasion and VEGF expression exhibited correlation with distant metastases (Table 2). Patients with distant metastases had significantly higher VEGF expression, mucinous subtype of adenocarcinoma, poorly differentiated tumors and lymphovascular invasion than patient without metastases ($p < 0.05$). To establish independent risk factors, four variables were identified as significant in univariate analysis. Additionally, they were tested in a multivariate Cox proportional hazards model. It revealed three factors to be associated with distant metastases: VEGF expression, mucinous adenocarcinoma and tumor differentiation (Table 3).

In patients with positive VEGF expression, DFS (Figure 1) and OS (Figure 2) were significantly worse than in negative ones (65% and 59%, respectively; *log-rank* test, $p < 0.05$).

Table 1
Clinicopathological characteristics of the patients

Variables	Number (%)
Sex	
male	102 (62.6)
female	61 (37.4)
Age (years)	
< 60	68 (41.7)
> 60	95 (58.3)
Distance from the anal verge (cm)	
< 5	30 (18.4)
5–10	75 (46.0)
> 10	58 (35.6)
Tumor size (cm)	
< 5	65 (39.9)
> 5	98 (60.1)
Type of operation	
abdominal resection	151 (92.6)
abdominoperineal resection	7 (4.2)
hartmann	5 (3.2)
Histological type	
adenocarcinoma	133 (81.6)
mucinous adenocarcinoma	30 (18.4)
Grade	
well differented	133 (81.6)
moderately differented	24 (14.7)
poorly differented	6 (3.7)
pT stage	
pT3a	29 (17.8)
pT3b	75 (46.0)
pT3c	44 (27.0)
pT3d	15 (9.2)
No of lymph nodes examined	
< 12	24 (14.7)
> 12	139 (85.3
Lymphovascular invasion	
positive	69 (42.3)
negative	94 (57.7)
VEGF expression	
positive	90 (55.2)
negative	73 (44.8)
Total	163 (100)

pT – primary tumor; VEGF – vascular endothelial growth factor.

Table 2

Univariate Cox-regression analysis of potential prognostic factors for distant metastases

Variables	Number of patients (n = 163)	Univariate analysis		
		hazard ratio	95% CI	*p*
Sex				
male	102	1.39	0.69–2.82	0.361
female	61			
Age (years)				
< 60	68	1.00	0.97–1.03	0.905
> 60	95			
Distance from the anal verge (cm)				
< 5	30	0.99	0.61–1.60	0.966
5–10	75			
> 10	58			
Tumor size (cm)				
< 5	65	0.92	0.45–1.87	0.811
> 5	98			
Histological type				
adenocarcinoma	133	3.40	1.65–7.03	0.001
mucinous adenocarcinoma	30			
Grade				
well differented	133	3.57	2.26–5.64	0.001
moderately differented	24			
poorly differented	6			
pT stage				
pT3a	29	1.17	0.78–1.75	0.462
pT3b	75			
pT3c	44			
pT3d	15			
No of lymph nodes examined				
< 12	24	0.66	0.27–1.62	0.367
> 12	139			
Lympho-vascular invasion				
positive	69	2.26	1.10–4.66	0.027
negative	94			
VEGF expression				
positive	90	6.35	2.22–18.17	0.001
negative	73			

pT – primary tumor; CI – confidence interval; VEGF – vascular endothelial growth factor.

Table 3

Multivariate Cox-regression analysis of potential prognostic factors for distant metastases

Variables	Number of patients (n = 163)	Multivariate analysis		
		hazard ratio	95% CI	*p*
Histological type				
adenocarcinoma	133	3.46	1.60-7.52	0.002
mucinous adenocarcinoma	30			
Grade				
well differented	133	3.02	1.79-5.08	0.001
moderately differented	24			
poorly differented	6			
Lympho-vascular invasion				
positive	69	2.02	0.93-4.42	0.076
negative	94			
VEGF expression				
positive	90	5.51	1.88-16.19	0.002
negative	73			

CI – confidence interval; VEGF – vascular endothelial growth factor.

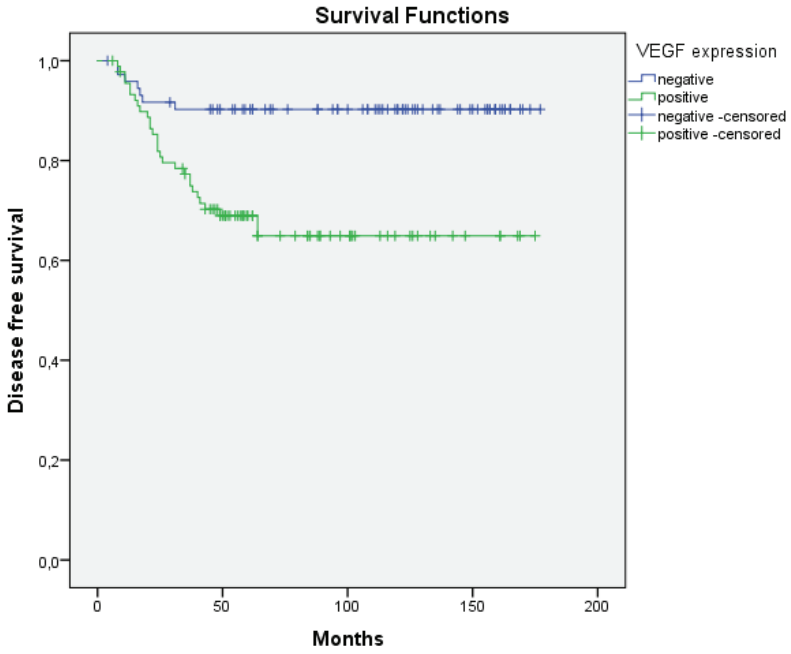

Fig. 1 – Kaplan-Meier`s curves of the disease-free survival of the patients according to the vascular endothelial growth factor (VEGF) expression.

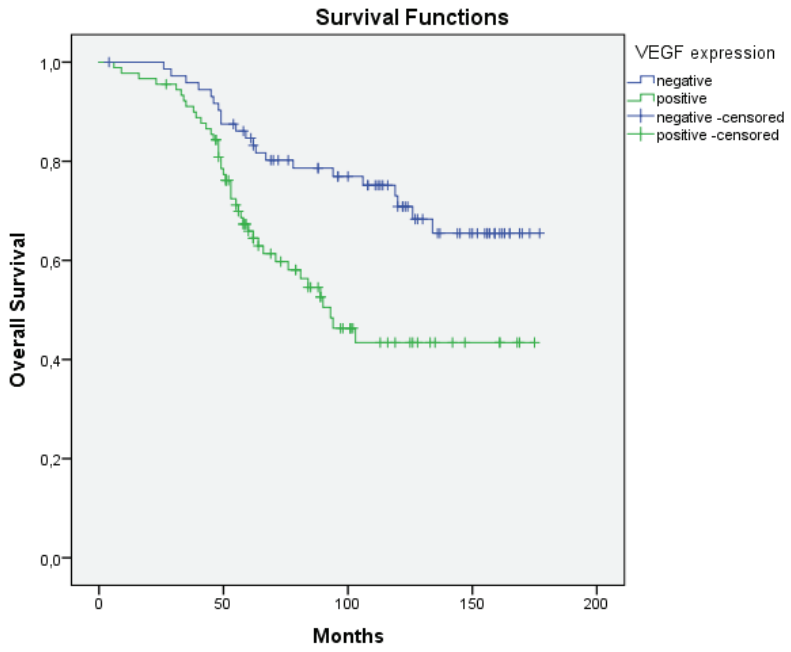

Fig. 2 – Kaplan-Meier`s curves of the overall survival of the patients according to the vascular endothelial growth factor (VEGF) expression.

Discussion

RC still presents major health problem. Developments in the field of surgery (introduction of TME) and neoadjuvant treatment led to the significant reduction in the percentage of LR and better quality of life of the affected patients [11, 12]. Local control and survival rates have been significantly improved. Major trials have reported that TME alone can reduce the local recurrence from 15%–20% to 4%–7% and improve the survival rate to 80%–85% for patients with the stage II disease [13].

Another fact is that screening programs, now widely implemented, have significant impact on the structure of operated patients. We have ever cases of early RC where we can expect favorable treatment results, but at a cost. Neoadjuvant treatment and TME surgery have certain downsides.

Namely, functional deficits, morbidity and mortality are inevitable in certain percent. Preoperative therapy can potentiate stated downsides [3, 8, 14].

Being aware of mentioned facts, we can conclude that certain population of patients would benefit from omitting chemoradiotherapy.

Improved preoperative staging can help us determine where it is safe to proceed only with surgical treatment of early RC. High-resolution magnetic resonance imeging (MRI) examination proved to be an excellent diagnostic tool which can help us in the decision where to omit neaodjuvant treatment [15]. T1 and T2 carcinomas can safely be treated with surgery alone [1]. But the population of patients with the T3N0 stage, circumferential resection margin (CRM) negative tumors can also benefit from this approach. There is no official consensus, or published guidelines, that clearly state that T3N0 RC deserves no surgery, but nowadays the majority of experts agree that surgery alone is sufficient treatment for this population of patients [1, 16].

Therefore, it is imperative to identify group of potential risk factors after TME in the cases of RC stages as the T3N0 disease in order to help further individualized strategy for those patients. There is a number of traditional clinical prognostic and risk factors [5, 17], but with no consensus reached which additional combination of biological factors would be most useful. In the mentioned group of patients, the risk of relapse is about 20% which makes standard adjuvant treatment unnecessary. There are attempts to select subgroup of the stage II patients using additional biomarkers where we should consider additional therapy.

In those efforts, a number of markers were investigated, among them one of more promising ones is VEGF [18, 19]. Angiogenesis is a key moment in tumor growth and in the development of metastatic potential and can be, in that context used, relatively reliable prognostic factor in patients with certain solid tumors. VEGF is 45kDa glycoprotein with the central role in tumor angiogenesis, influencing other proangiogenic factors and their inhibition suppresses tumor growth. There are different VEGF protein isoforms having subunit polypeptides of 121, 145, 165, 189 or 206 amino acid residues. $VEGF_{165}$ is the predominant molecular species, but transcripts encoding $VEGF_{189}$ are also commonly found in cells expressing the VEGF gene. $VEGF_{145}$ is the major splice variant in several tumor cell lines originating from the female reproductive organs. In contrast, $VEGF_{206}$ is a rare form. The splice variants differ in their bioavailability. It would be interesting to investigate prognostic and predictive value of each individual isoform in CRC patients, which would be a topic for further research [9, 10].

VEGF expression together with some standard histopathological tumor features could give us enough information to identify subgroup of the high risk stage II RCs.

Our study confirmed well known facts that mucinous component and poor differentiation of the tumor means unfavorable prognosis for RC patients in terms of the development of distant metastases. However, real biological aggressiveness of these tumors is often difficult to assess. In this group of tumors we can find signet ring cell carcinomas as well as those with partial mucin production. (i.e. mucinous and mixed neuroendocrine carcinomas) [20, 21]. Histological grade of the tumor on the other hand, can be subjective with considerable inter observer variations [20].

In our study, in 55.2% analyzed samples, higher VEGF expression was found and was associated with the development of the distant metastases. Five year DFS in VEGF positive patients was 65% and in VEGF negative group 90%. OS was also significantly affected when comparing VEGF positive and negative cases (59% and 80% respectively). There are studies that reached similar conclusion [22–24], but majority of studies found significant association of VEGF expression and more advanced stages of the disease (stage III and IV) [18, 19]. High VEGF expression is associated with poor prognosis and advanced stage of the disease, but its real prognostic significance in patients with CRC is still unclear, especially in the early stage. One of the reasons for this can be pathologist bias, nonstandardized staining protocols and scoring systems as well as the differences in analyzed materials (stage of the disease, number of participants...).

Finally, despite reaching statistically significant results, we must state that our study had limitations (stage of the disease, only RC patients and retrospective nature).

Conclusion

VEGF can be potentially used as prognostic factor in patients with T3N0 RCs. Combined with standard clinical and pathological prognostic factors it can help us identify the group of patients with poor prognosis who can be candidates for adjuvant treatment and more aggressive follow-up protocol. Nevertheless, more prospective multicentric studies are needed in order to finally establish the real role of VEGF as a prognostic factor in patients with early RC.

REFERENCES

1. *Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A,* et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017; 28(Suppl 4): iv22–iv40.
2. *Wong SJ, Moughan J, Meropol NJ, Anne PR, Kachnic LA, Rashid A,* et al. Efficacy endpoints of radiation therapy group protocol 0247: A randomized, phase 2 study of neoadjuvant radiation therapy plus concurrent capecitabine and irinotecan or capecitabine and oxaliplatin for patients with locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 2015; 91(1): 116–23.
3. *Song JH, Jeong JU, Lee JH, Kim SH, Cho HM, Um JW, et al.* Preoperative chemoradiotherapy versus postoperative chemoradiotherapy for stage II-III resectable rectal cancer: A meta-analysis of randomized controlled trials. Radiat Oncol J 2017; 35(3): 198–207.
4. *Mejri N, Dridi M, Labidi S, El Benna H, Daoud N, Boussen H.* Annual hazard rate of relapse of stage II and III colorectal cancer after primary therapy. Clin Transl Oncol 2017; 19(12): 1524–30.

5. *Nissan A, Stojadinovic A, Shia J, Hoos A, Guillem JG, Klimstra D,* et al. Predictors of recurrence in patients with T2 and early T3, N0 adenocarcinoma of the rectum treated by surgery alone. J Clin Oncol 2006; 24(25): 4078–84.
6. *Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK,* et al. Molecular biomarkers for the evaluation of colorectal cancer: Guideline summary from the American society for clinical pathology, college of American pathologists, association for molecular pathology, and American society of clinical oncology. J Oncol Pract 2017; 13(5): 1453–86.
7. *Kim JW, Kim YB, Choi JJ, Koom WS, Kim H, Kim NK,* et al. Molecular markers predict distant metastases after adjuvant chemoradiation for rectal cancer. Int J Radiat Oncol Biol Phys 2012; 84(5): e577–84.
8. *Van Gijn W, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T,* et al. Dutch Colorectal Cancer Group. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol 2011; 12(6): 575–82.
9. *Hanrahan V, Currie MJ, Gunningham SP, Morrin HR, Scott PA, Robinson BA,* et al. The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. J Pathol 2003; 200(2): 183–94.
10. *Martins SF, Garcia EA, Luz MAM, Pardal F, Rodrigues M, Filho AL.* Clinicopathological correlation and prognostic significance of VEGF-A, VEGF-C, VEGFR-2 and VEGFR-3 expression in Colorectal cancer. Cancer Genomics Proteomics 2013; 10(2): 55–67.
11. *Kapiteijn E, Marijnen CA, Nagtegaal ID, Putter H, Steup WH, Wiggers T,* et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 2001; 345(9): 638–46.
12. *Heald RJ, Husband EM, Ryall RD.* The mesorectum in rectal cancer surgery"the clue to pelvic recurrence? Br J Surg 1982; 69(10): 613–6.
13. *Kulu Y, Tarantino I, Billeter AT, Diener MK, Schmidt T, Büchler MW,* et al. Comparative Outcomes of Neoadjuvant Treatment Prior to Total Mesorectal Excision and Total Mesorectal Excision Alone in Selected Stage II/III Low and Mid Rectal Cancer. Ann Surg Oncol 2016; 23(1): 106–13.
14. *Glimelius B, Grönberg H, Järhult J, Wallgren A, Cavallin-Ståhl E.* A systematic overview of radiation therapy effects in rectal cancer. Acta Oncol 2003; 42(5–6): 476–92.
15. *Taylor FG, Quirke P, Heald RJ, Moran BJ, Blomqvist L, Swift IR,* et al. Preoperative magnetic resonance imaging assessment of circumferential resection margin predicts disease-free survival and local recurrence: 5-Year follow-up results of the MERCURY Study. J Clin Oncol 2014; 32(1): 34–43.
16. *Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K,* et al. Esmo consensus guidelines for management of patients with colon and rectal cancer. A personalized approach to clinical decision making. Ann Oncol 2012; 23(10): 2479–516.
17. *Nikberg M, Chabok A, Letocha H, Kindler C, Glimelius B, Smedh K.* Lymphovascular and perineural invasion in stage II rectal cancer: a report from the Swedish colorectal cancer registry. Acta Oncol 2016; 55(12): 1418–24.
18. *Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R,* et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer 2006; 94(12): 1823–32.
19. *Wang Y, Yao X, Ge J, Hu F, Zhao Y.* Can vascular endothelial growth factor and microvessel density be used as prognostic biomarkers for colorectal cancer? A systematic review and meta-analysis. ScientificWorldJournal 2014; 2014: 102736.
20. *Gunther K, Dworak O, Remke S, Pfluger R, Merkel S, Hohenberger W, et al.* Prediction of distant metastases after curative surgery for rectal cancer. J Surg Res 2002; 103(1): 68–78.
21. *Whittaker MA, Carr NJ, Midwinter MJ, Badham DP, Higgins B.* Acinar morphology in colorectal cancer is associated with survival but is not an independent prognostic variable. Histopathology 2000; 36(5): 439–42.
22. *Zafirellis K, Agrogiannis G, Zachaki A, Gravani K, Karameris A, Kombouras C.* Prognostic significance of VEGF expression evaluated by quantitative immunohistochemical analysis in colorectal cancer. J Surg Res 2008; 147(1): 99–107.
23. *Cascinu S, Staccioli MP, Gasparini G, Giordani P, Catalano V, Ghiselli R,* et al. Expression of vascular endothelial growth factor can predict event-free survival in stage II colon cancer. Clin Cancer Res 2000; 6(7): 2803–7.
24. *Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR,* et al. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. Arch Surg 1997; 132(5): 541–6.

Received on January 20, 2018.
Revised on March 23, 2018.
Accepted on April 5, 2018.
Online First April, 2018.