Use of the Systemic Immune Inflammation Index, TNM classification, and CEA in pre- and post- prognostic evaluation of sporadic colorectal cancer

Ann. Ital. Chir., 2022 93, 3: 319-324
pii: S0003469X22033528

Hakan Uzunoğlu*, Mehmet Onur Gül**, Umut Kina*, Meryem Günay Gürleyik*

**Department of General Surgery, Haydarpaşa Numune Training and Research Hospital, Istanbul, Turkey.*
***Department of General Surgery, Malatya Training and Research Hospital, Malatya, Turkey*

Use of the Systemic Immune Inflammation Index, TNM classification, and CEA in pre- and post-prognostic evaluation of sporadic colorectal cancer.

AIM: *The aim of this study was to investigate the correlation between preoperative systemic immune inflammatory index (SII) and carcinoembryonic antigen (CEA) and postoperative lympho-vascular invasion and TNM stage in patients with colorectal carcinoma.*
MATERIAL AND METHODS: *A total of 153 patients with colorectal cancer admitted to our tertiary hospital between 2014 and 2018 were included in the study.*
RESULTS: *While 71.2% of the patients had low preoperative CEA values, 28.8% had high preoperative CEA values. Lymphovascular invasion rate was found to be significantly higher in patients with high CEA levels than those in low levels (70.4% vs. 39.4%, p=0.002). The percentage of patients with lymphovascular invasion with a high SII (60.0%) was significantly higher than those without lymphovascular invasion (p=0.015).*
CONCLUSION: *In our study, serum CEA and SII index values were found to provide critical information in terms of showing lympho-vascular invasion, which is considered as an independent "bad" prognostic factor in patients with colorectal carcinoma. In conclusion, we consider that CEA and SII index values can be used to determine the prognosis of patients with colorectal cancer.*

KEY WORDS: Carcinoembryonic antigen, Colorectal carcinoma, Microsatellite, lymphovascular invasion, TNM

Introduction

Colorectal carcinomas are one of the most common cancers in the world. It is the second most common cancer in women and the third among men [1,2]. Clinicians use some indicators pre- and postoperatively to predict the prognosis of these cancers. Carcinoembryonic antigen (CEA) is a common prognostic factor used to monitor the prognosis of colorectal carcinoma. CEA, which is first described in 1965, is an important regulatory protein involved in intracellular adhesion and aggregation. It also provides valuable information regarding the success of the treatment and presence of the recurrences [3]. TNM staging is taken into consideration in both treatment and estimation of the prognosis. However, it is reported that the prognosis of patients in the same stage occasionally shows heterogeneity in the clinic presentation [4,5].

Despite all tools including clinical staging, pathological evaluation and serum biomarker levels, used to designate the prognosis in colorectal cancer patients, inadequacies in predicting prognosis reliably led researchers to search for cheaper and simpler prognostic indicators. In recent years, it has been suggested that there is a relationship between tumor carcinogenesis and host inflammatory response, which may play a role in the progression and metastasis of cancer cells [2,6,7]. It is stated that the systemic immune inflammation index (SII) calculated by

Pervenuto in Redazione Maggio 2020. Acettato per la pubblicazione Giugno 2020.
Correspondence to: Hakan Uzuno lu, M.D., Department of General Surgery, Haydarpa a Numune Training and Research Hospital, Istanbul, Turkey (e-mail: drhakanuzunoglu@gmail.com)

using the systemic inflammation parameters such as neutrophil, platelet and lymphocyte can be used in the prediction of prognosis in some solid malignancies including colorectal carcinoma [8].
The aim of this study was to investigate the correlation between preoperative SII and carcinoembryonic antigen (CEA) and postoperative lymphovascular invasion and TNM stage in patients with colorectal carcinoma.

Methods

Patients

A total of 153 elective, resectable, sporadic colorectal cancer patients admitted to our tertiary hospital between 2014 and 2018 were included in the study. Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board and was performed in accordance with the Helsinki declaration. Patients with a history of abdominal surgery, emergency cases with signs of oppression, and previous tumor history and those with unresectable tumors due to diffuse metastasis were excluded from the study. Peripheral blood samples were obtained for measuring preoperative and postoperative CEA levels and for complete blood counts. Lymphovascular invasion positivity and TNM stage were determined for each patient. Patients with stage 1-2 in the TNM classification were considered to be the low stage, and those with a 3-4 TNM stage were regarded as high stage [9].

Tests

Blood samples obtained from patients were tested for CEA (Abbott, Germany) and hemogram levels (Midray, China) according to the recommendations of the kit manufacturer. The CEA threshold value was accepted as 5 ng/Ml [9]. The following formula was used to calculate the SII: SII = neutrophil count x platelet count/lymphocyte count. The threshold value for the SII was accepted as 340 ng/mL [8].

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 software (IBM SPSS, Chicago, IL, USA). Descriptive data were given as numbers and percentages. The suitability of the parameters to normal distribution was evaluated by the Shapiro Wilk test. Comparisons between the groups in terms of categorical variables were made by Chi-square test and Fisher's Exact Test and Continuity (Yates) Correction was used. Results were evaluated in 95% confidence interval and $p < 0.05$ was considered significant.

Results

A total of 153 patients were enrolled in the study (mean age 67.68 ± 11.09 years, 62 % male).
While 71.2% of the patients had low preoperative CEA values (<5 ng/mL), 28.8% had high preoperative CEA values (>5 ng/mL). 16.3% of the patients had TNM stage 1, 43.1% had TNM stage 2, 35.3% had TNM stage 3 and 5.2% had TNM stage 4 tumors. The tumor T stage was 1 in 4.6% of the patients, 2 in 13.7%, 3 in 69.9% and 4 in 11.8% of the subjects. 59.5% of the patients had a low stage (TNM Stage 1-2), and 40.5% had high stage (TNM Stage 3-4) tumors. The lymph node stage was 0 in 58.2%, 1 in 26.1% and was 2 in 15.7% of the subjects. Microsatellite instability was observed in 33.3%, and lymphovascular invasion and perineural invasion was noted in 48.7%, and 22.2% of the subjects, respectively. SII ranged between 8.97 and 20045.9 with a mean value of 1370.04 ± 2165.57 and a median value of 764.6. When the patients were classified according to the SII, 49.7%had low SII, and 50.3% had high SII. Tumor differentiation was low in 5.2%, moderate in 83%, and good in 11.8% (Table I). Lymphovascular invasion was detected in 70.4% of

Table I - *Distributions of the variables of the study.*

Variables		n	%
Preop CEA (threshold: 5 ng/mL)	Low	109	71.2
	High	44	28.8
TNM stage (n=153)	1	25	16.3
	2	66	43.1
	3	54	35.3
	4	8	5.2
Tumor T stage (n=153)	1	7	4.6
	2	21	13.7
	3	107	69.9
	4	18	11.8
TNM stage (n=153)	Low (1-2)	91	59.5
	High (3-4)	62	40.5
Lymph node metastasis (n=153)	0	89	58.2
	1	40	26.1
	2	23	15
	3	1	0.7
Microsatellite instability (n=153)	Present	51	33.3
	Absent	103	66.9
Lymphovascular invasion (n=153)	Present	74	48.4
	Absent	79	51.6
Perineural invasion (n=153)	Present	34	22.2
	Absent	119	77.8
High SII (n=153) (threshold: 340)	Low	76	49.7
	High	77	50.3
Location (n=153)	Right	38	24.8
	Left	66	43.1
	Right-Left	2	1.3
	Rectum	46	30.1
	Total	1	0.7
Differentiation (n=153)	Bad	8	5.2
	Middle	127	83
	Good	18	11.8

patients with high preoperative serum CEA levels and 39.4% of patients with low CEA levels, and lymphovascular invasion rate was found to be significantly higher in patients with high CEA levels compared to those with low CEA levels (p=0.002). The sensitivity and specificity of high preoperative CEA was 41.9% to identify the subjects with lymphovascular invasion were 41.9%, and 82.1%, respectively. The positive and negative predictive values for a high CEA to determine the lymphovascular invasion 68.9%, and59.8%, respectively, and the accuracy was 62.8%. The percentage of patients with lymphovascular invasion with high SII (60.8%) was significantly higher than those without lymphovascular invasion (p=0.015). The sensitivity and specificity of high SII to identify the subjects with lymphovascular invasion 60.8%, and 59.0%, respectively. The positive and negative predictive values of SII to determine the lymphovascular invasion 58.4%, and 61.3%, respectively, and the accuracy were 59.9%. Patients with lymphovascular invasion had higher SII and CEA values (28.4%) than those without lymphovascular invasion (6.4%) (p=0.001). The sensitivity and specificity of the combination of the high SII and high CEA for identifying lymphovascular invasion was 28.4%, and 93.6%, respectively, whereas the positive predictive value, negative predictive value and accuracy were 80.8%, 58.0%, and 61.8%, respectively (Table II)

.

TNM staging was found to be similar in patients with high preoperative CEA levels and in those with low preoperative CEA levels (p=0.174). In addition, no significant difference was found between the patients with high and low SII with respect to the TNM stage (p=0.114). The specificity, and positive and negative predictive values for CEA to determine the TNM stage were 74.73%, 48.89%, and 62.96%, respectively, and the accuracy was 52.82%. The sensitivity, specificity, and positive and negative predictive values for SII to determine the TNM stage were 58.06%, 54.95%, 46.75%, and 65.79%, respectively, and the accuracy was 56.21%. There were no significant differences between the low and high TNM stage in terms of the distribution rates of high SII and CEA (p> 0.082). The sensitivity, specificity and positive and negative predictive values of the combination of the high SII and high CEA for identifying subjects with a high TNM stage were 24.19%,87.91%, 57.69%, and62.99%, respectively, and the accuracy was 62.09% (Table III).

No significant differences were found between patients with and without lymphovascular invasion in terms of the presence of microsatellite instability (p=0.567). There were no significant differences in terms of TNM stage elevation distribution rates between stable and unstable microsatellite (p=0.415) (Table IV).

TABLE II - *The performance of SII and CEA in identification of the subjects with lymphovascular invasion.*

		Lymphovascular invasion				
		Present	%	Absent	%	p
HighCEA	Low	43	39.4	66	60.6	0.002[1]
	High	31	70.4	13	29.6	
High SII	Low	29	38.7	46	61.4	0.015[2]
	High	45	60.0	30	40.0	
High SII and High CEA	Low	21	80.8	5	19.3	0.001[1]
	High	53	41.7	74	58.3	

CEA: Carcinoembryonic antigen, SII: Systemic immune inflamatory index.
[1]Continuity (yates) correction
[2]Chi square test

TABLE III - *The performance of SII and CEA in determination of TNM stage.*

		TNM stage				
		Low (1-2)	(%)	High (3-4)	%	p
High CEA	Low	68	63	40	37.1	0.174[1]
	High	23	51.2	22	48.9	
High SII	Low	50	65.8	26	34.3	0.114[1]
	High	41	53.3	36	46.8	
High SII and high CEA	High	11	42.4	15	57.7	0.082[2]
	Not high	80	63	47	37.1	

CEA: Carcinoembryonic antigen, SII: Systemic immune inflamatory index.
[1]Chi square test
[2]Continuity (yates) correction

TABLE IV - *Evaluation of the presence of lymphovascular invasion and TNM stage elevation among microsatellite groups.*

		Microsatellite instability				
		Present	%	Absent	%	p
Lymphovascular invasion	Present	27	35.2	48	64.9	0.567
	Absent	24	30.8	54	69.3	
TNM stage	Low	28	30.8	63	69.3	0.415
	High	23	37.1	39	63	

Chi square test

Discussion

Colorectal carcinoma is a common type of cancer, which is associated with high morbidity and mortality rates. Early diagnosis may improve prognosis and increase survival in patients with colorectal carcinoma. Therefore, predicting the prognosis in subjects with colorectal carcinoma is critical. Many prognostic indicators continue to be investigated in this regard [10-12]. In our study, the prognostic value of the SII and CEA levels in colorectal patients were evaluated. In this study, TNM stage

and lymphovascular invasion positivity and microsatellite instability, which are independent prognostic indicators in colorectal carcinoma were used in the assessment of the prognosis.

CEA is believed to play a significant role in tumor invasion and metastasis. It can be found at low levels in the embryonic and fetal small intestine or adult human cells. However, serum levels increase in 90% of primary colorectal carcinomas [13-18]. Serum CEA level has been used for decades as a tumor marker for detecting colorectal carcinomas and determining prognosis in patients with colorectal carcinomas. Despite its prognostic value, it remains controversial due to its low sensitivity and suspicious effects on mortality [10]. In addition, high CEA levels observed in a variety of different types of malignancies such as stomach, pancreas and lung cancers decrease the specificity of CEA for diagnosing colorectal carcinomas. Therefore, it is recommended to be used in patient follow-up rather than early diagnosis [10,12,13]. Sørensen et al. conducted a meta-analysis of 42 studies investigating the sensitivity and specificity of CEA values for detecting colorectal carcinoma [10]. They stated that different values between 2.5 ng/mL and 40 ng/mL were used as the serum CEA threshold in their analysis. However, 27 of the studies included in their analysis reported that 5 ng/mL was an acceptable threshold. They stated that sensitivity rates were reported to be between 43-86%, and specificity rates were reported to be between 66-98% in studies where the threshold value was accepted as 5 ng/mL. In our study, the CEA cut-off value was, therefore, accepted as 5 ng/ml to determine the relationship between CEA and patients' prognosis. In our study, lymphovascular invasion was detected in 70.4% of patients with high preoperative serum CEA levels and 39.4% of patients with low CEA levels, and lymphovascular invasion rate was found to be significantly higher in patients with high CEA levels. Lim et al. [19] reported that lymphovascular invasion positivity is an independent predictor of poor prognosis and tumor aggression in sporadic colorectal cancer. With this in mind, we speculate that preoperative CEA level can provide valuable information regarding the prognosis of patients with colorectal carcinoma.

Several scoring systems have been introduced to predict the prognosis in colorectal carcinomas [8,20]. Neutrophil lymphocyte ratio and serum lactate dehydrogenase levels have a predictive and prognostic role in many disorders, including colorectal carcinomas. With the combination of these markers, a scoring system called the colonic inflammatory index was established [20]. Chen et al. used the systemic immune-inflammation index for the first time in their study with 1383 patients with colorectal carcinomas [8].They stated that the SII, which is calculated by dividing the product of serum neutrophil and platelet numbers by lymphocyte number, is a very powerful tool for predicting survival rate in patients with colorectal carcinoma. They also reported that the SII helps identify high-risk patients among those with the same TNM stage. Chen et al. found the sensitivity and specificity of SII > 340 for identifying patients with colorectal carcinomas who are under a high-risk for mortality as 85.7%, and 52.4%, respectively. Tao et al. found that mean SII was significantly higher in patients with colorectal carcinoma compared to healthy subjects, and reported a significant correlation between SII and serum CEA levels [9]. Therefore, we used the SII index in our study and accepted the threshold as 340. In our study, the rate of lymphovascular invasion was significantly higher in patients with high SII compared to patients with low SII. According to this data, preoperative SII level provides valuable information in terms of patient's prognosis. In our study, lymphovascular invasion was found as high as 80.8% in patients with high preoperative CEA level and SII. Combined use of SII and CEA level is more useful in the detection lymphovascular invasion than the use of SII and CEA separately.

Although different markers have been investigated in determining the prognosis of patients with colorectal carcinomas, TNM staging still remains the most reliable one among them. It has been reported that there are some limitations in the TNM staging system based on tumor invasion depth and lymph node and distant organ metastasis. In addition, TNM staging cannot provide as much information about the course of the disease as some new markers[21]. It has been stated that the use of TNM staging may be sufficient for guiding the management, but it is emphasized that TNM staging does not adequately define some of the risk factors affecting the prognosis in colorectal carcinoma [21]. Huh et al. reported a significant correlation between high CEA levels (> 5 ng/mL) and TNM stages in their study conducted on 474 colorectal carcinoma patients with metastasis [22]. Lee et al. reported that TNM stage was significantly higher in patients with high preoperative CEA levels [23]. However, Topdagi et al. did not find a significant difference in TNM stages among patients with colorectal carcinoma and a CEA value above or below 5 ng/mL [18]. In our study, TNM staging was similar in patients with high and low preoperative CEA levels. In addition, there were no significant differences in TNM stages between patients with high and low SII. These findings indicate that CEA and SII levels cannot provide definitive information regarding the TNM stage of the subjects with colorectal carcinomas.

Microsatellite instability (MSI), which is the somatic accumulation of length changes in repetitive DNA sequences called microsatellite, is frequently seen in hereditary and sporadic colorectal cancer [24]. It has been reported that colorectal cancers may occur in two different mutational ways, such as microsatellite instability or chromosomal instability [25]. Gryfe et al. demonstrated microsatellite instability in 17% of patients with colorectal carcinoma and found that microsatellite instability significant survival advantage in colorectal carcinoma

independent of all prognostic factors, including tumor stage [25].In our study, microsatellite instability was detected in 33.3% of the study population. In addition, no significant difference was found between patients with and without lymphovascular invasion with regard to the presence of microsatellite instability. Also, there were no significant differences in the rates of microsatellite instability between subjects with high and low TNM stages. These results indicate that the detection of lymphovascular invasion and TNM stages are not informative in terms of the presence of microsatellite instability.

Conclusions

In our study, serum CEA level and SII were found to provide critical information in terms of lymphovascular invasion, which, is considered as an independent "bad" prognostic factor in patients with colorectal carcinoma. We believe that CEA and SII values can be used to determine the prognosis of patients with colorectal cancer.

Riassunto

Lo scopo di questo studio è quello di indagare la correlazione tra indice immunitario infiammatorio sistemico preoperatorio (SII) e antigene carcinoembryonic (CEA) con l'invasione linfo-vascolare postoperatoria e stadio TNM in pazienti con carcinoma del colon-retto.
Sono stati inclusi nello studio 153 pazienti con carcinoma del colon-retto ricoverati nel nostro ospedale terziario tra il 2014 e il 2018.
RISULTATI: Mentre il 71,2% dei pazienti presentava bassi valori CEA preoperatori, il 28,8% presentava alti valori CEA preoperatori. Il tasso di invasione linfovascolare è risultato significativamente più alto nei pazienti con livelli elevati di CEA rispetto a quelli con livelli bassi (70,4% vs. 39,4%, p = 0,002). La percentuale di pazienti con invasione linfovascolare con SII elevato (60,0%) era significativamente più alta rispetto a quelli senza invasione linfovascolare (p = 0,015).
In conclusione nel nostro studio, i valori sierici di indice CEA e SII hanno dimostrato di fornire informazioni critiche in termini di invasione linfo-vascolare, considerata un fattore prognostico "cattivo" indipendente nei pazienti con carcinoma del colon-retto.
In conclusione, riteniamo che i valori dell'indice CEA e SII possano essere utilizzati per determinare la prognosi dei pazienti con carcinoma del colon-retto.

References

1. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ: *Colorectal carcinoma: A general overview and future perspectives.* In *Colorectal Cancer*. Int J Mol Sci, 2017; 19;18(1). pii: E197. doi: 10.3390/ijms18010197.

2. Puerta-García E, Cañadas-Garre M, Calleja-Hernández MÁ: *Molecular biomarkers in colorectal carcinoma.* Pharmacogenomics, 2015; 16:1189-222. doi: 10.2217/PGS.15.63.

3. Duffy MJ: *Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful?* Clin Chem, 2001; 47:624-30.

4. Peparini N, Beyond T, N, M: *The impact of tumor deposits on the staging and treatment of colorectal and gastric carcinoma*. Surg Oncol, 2018; 27:129-37. doi: 10.1016/j.suronc.2018.02.007.

5. Li J, Yi CH, Hu YT, Li JS, Yuan Y, Zhang SZ, et al.: *TNM Staging of Colorectal Cancer Should be Reconsidered According to Weighting of the T Stage: Verification Based on a 25-Year Follow-Up*. Medicine (Baltimore), 2016;95:e2711. doi: 10.1097/ MD. 0000000000002711.

6. Lucas C, Barnich N, Nguyen HTT: *Microbiota, inflammation and colorectal cancer*. Int J Mol Sci, 2017; 20:18(6). pii: E1310. doi: 10.3390/ijms18061310.

7. Chen J, Pitmon E, Wang K: Mic*robiome, inflammation and colorectal cancer*. Semin Immunol, 2017; 32:43-53. doi: 10.1016/ j.smim.2017.09.006.

8. Chen JH, Zhai ET, Yuan YJ, Wu KM, Xu JB, Peng JJ, et al.: *Systemic immune-inflammation index for predicting prognosis of colorectal cancer*. World J Gastroenterol, 2017; 14:23:6261-72. doi: 10.3748/wjg.v23.i34.6261.

9. Tao MY, Wang ZH, Zhang MH, Ma TH, Yang XZ, Wu SN, Chen XF, Wang HG: *Prognostic value of the systematic immune-inflammation index among patients with operable colon cancer: A retrospective study.* Medicine (Baltimore), 2018;97:e13156. doi: 10.1097/MD.0000000000013156.

10. Sørensen CG, Karlsson WK, Pommergaard HC, Burcharth J, Rosenberg J: *The diagnostic accuracy of carcinoembryonic antigen to detect colorectal cancer recurrence. A systematic review.* Int J Surg, 2016; 25:134-44. doi: 10.1016/j.ijsu.2015.11.065.

11. Colloca G, Venturino A, Vitucci P: *Pre-treatment carcinoembryonic antigen and outcome of patients with rectal cancer receiving neo-adjuvant chemo-radiation and surgical resection: A systematic review and meta-analysis*. Med Oncol, 2017; 34:177. doi: 10.1007/s12032-017-1037-8.

12. Lopez NE, Peterson CY*: Advances in biomarkers: Going beyond the carcinoembryonic antigen*. Clin Colon Rectal Surg, 2016; 29:196-204. doi: 10.1055/s-0036-1584289.

13. Zhang SY, Lin M, Zhang HB: *Diagnostic value of carcinoembryonic antigen and carcinoma antigen 19-9 for colorectal carcinoma.* Int J Clin Exp Pathol, 2015; 1:9404-9.

14. Kelleher M, Singh R, O'Driscoll CM, Melgar S: *Carcinoembryonic antigen (CEACAM) family members and Inflammatory Bowel Disease*. Cytokine Growth Factor Rev, 2019; 47:21-31. doi: 10.1016/j.cytogfr.2019.05.008.

15. Tong G, Xu W, Zhang G, Liu J, Zheng Z, Chen Y, et al.: *The role of tissue and serum carcinoembryonic antigen in stages I to III of colorectal cancer. A retrospective cohort study.* Cancer Med, 2018; 7:5327-38. doi: 10.1002/cam4.1814.

16. Vuijk FA, Hilling DE, Mieog JSD, Vahrmeijer AL: *Fluorescent-guided surgery for sentinel lymph node detection in gastric cancer and*

carcinoembryonic antigen targeted fluorescent-guided surgery in colorectal and pancreatic cancer. J Surg Oncol, 2018; 118:315-23. doi: 10.1002/jso.25139.

17. Campos-da-Paz M, Dórea JG, Galdino AS, Lacava ZGM, de Fatima Menezes Almeida Santos M: *Carcinoembryonic Antigen (CEA) and Hepatic Metastasis in Colorectal Cancer: Update on Biomarker for Clinical and Biotechnological Approaches.* Recent Pat Biotechnol, 2018; 12:269-79. doi: 10.2174/1872083126661807311044244.

18. Topdagi O, Timuroglu A: *Evaluation of the Relationship between Carcinoembryonic Antigen and TNM Stage in Colorectal Cancer.* Eurasian J Med, 2018; 50:96-8. doi: 10.5152/eurasianjmed. 2018.17093.

19. Lim SB, Yu CS, Jang SJ, Kim TW, Kim JH, Kim JC: *Prognostic significance of lymphovascular invasion in sporadic colorectal* cancer. Dis Colon Rectum, 2010;53:377-384. doi: 10.1007/ DCR.0b013e3181cf8ae5.

20. Casadei-Gardini A, Scarpi E, Ulivi P, Palladino MA, Accettura C, Bernardini I, et al.: *Prognostic role of a new inflammatory index with neutrophil-to-lymphocyte ratio and lactate dehydrogenase (Sİİ: ColonInflammatory Index) in patients with metastatic colorectal cancer: results from the randomized Italian Trial in Advanced Colorectal Cancer (ITACa) study*. Cancer Manag Res, 2019; 11:4357-69. doi: 10.2147/CMAR.S198651.

21. Sreekumar R, Harris S, Moutasim K, DeMateos R, Patel A, Emo K, et al.: *Assessment of Nuclear* ZEB2 as *a Biomarker for ColorectalCancer Outcome and TNM Risk Stratification*. JAMA Netw Open, 2018; 5:1.e183115. doi: 10.1001/jamanetworkopen.

22. Ahmed Farag AF, Elbarmelgi MY, Azim HA, Abozeid AA, Mashhour AN*: TNMF versus TNM in staging of colorectal cancer.* Int J Surg, 2016; 27:147-50. doi: 10.1016/j.ijsu.2016.01.087.

23. Huh JW, Oh BR, Kım HR, Kim YJ: *Preoperative carcinoembryonic antigen level as an independent prognostic factor in potentially curative colon cancer*. J Surg Oncol, 2010; 101: 396-400. doi: 10.1002/jso.21495.

24. Westdorp H, Fennemann FL, Weren RD, Bisseling TM, Ligtenberg MJ, Figdor CG, et al.: *Opportunities for immunotherapy in microsatellite instable colorectal cancer.* Cancer Immunol Immunother, 2016; 65:1249-259. doi: 10.1007/s00262-016-1832-7.

25. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al.: *Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer.* N Engl J Med, 2000; 342:69-77. doi: 10.1056/NEJM200001133420201.