Morbidity and long-term results in patients with wild and mutant type Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations undergoing colorectal cancer surgery

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BACKGROUND: *In colorectal cancer (CRC), the mutation of the K(N)RAS gene has a significant impact on the clinical course, and is associated with a negative prognosis. We aim to present the morbidity and long-term results in patients with wild/mut-K(N)RAS, undergoing CRC surgery.*
METHODS: *A total of 116 patients who underwent surgery for colorectal cancers with wild/mut-K(N)RAS were included in this retrospective study. The patients were divided into two groups: wild-K(N)RAS patients (Group 1) and mutant-K(N)RAS patients (Group 2). Results were evaluated for clinical, operative, morbidity and long-term survival outcomes.*
MATERIALS AND METHODS: *The highest surgical site infection (SSI) rate (OR=140.339)(4.303-4581.307)(P=0.005) was seen in patients given Bevacizumab during neoadjuvant treatment. Meanwhile, the SSI site infection rate was at its lowest in cases where minimally invasive surgery was preferred (OR=0.062)(0.006-0.628)(P=0.019). In addition, the overall median survival rate for the total cohort was 38±3.1 (31-44) months. Multivariate analysis showed that CEA (>5ng/mL)(HR 2.94)(1.337-6.492))(P=0.007); tumor stage (P=0.034), T(T4) stage (HR 1.91)(1.605-252.6)(P=0.02); metastasectomy/ablation (HR 0.19)(0.077-0.520)(P=0.001); the number of removed metastatic lymph nodes (HR 1.08)(1.010-1.155)(P=0.025); tumor implant or nodule (HR 2.71)(1.102-6.706)(P=0.03); curative resection (HR 2.40)(0.878-6.580)(P=0.042) to be factors affecting the overall survival rate.*
CONCLUSION: *Treatment with Bevacizumab during the neoadjuvant period in mut-K(N)RAS cases, surgical technique and complications of Grade 3 or higher are risk factors for SSI on morbidity in patients with mut/wild-K(N)RAS undergoing colorectal cancer surgery. Moreover, CEA (>5ng/mL), tumor stage, T stage, metastasectomy/ablation, the number of removed metastatic lymph nodes, tumor implant/nodule and curative resection are risk factors on the overall survival rate.*

KEY WORDS: Bevacizumab, Colorectal cancer, K(N)RAS mutation, Morbidity, Mortality

Introduction

Colorectal cancer (CRC) is currently the third most common cancer and third leading cause of cancer-related deaths in the United States [1]. The carcinogenesis of the CRC is multifactorial. Genome integrity or genetic instability, including chromosomal instability, microsatellite instability and epigenetic changes are some of the most important factors in carcinogenesis [2,3]. Fearon and Vogelstein's model, known as the "adenoma-carcinoma sequence", has offered a clear definition of chromosomal instability for carcinogenesis of the CRC since the 1990s [4]. Kirsten rat sarcoma (K-Ras) (produced by the gene KRAS) is a proto-oncogene protein which facilitates cell proliferation, differentiation, angiogenesis, invasion and

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survival in late adenoma. Produced as a result of downstream effects of the epidermal growth factor receptor (EGFR); as the EGF ligand is bound to its receptor, K-Ras downstream signaling is activated along the mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) cellular proliferation pathways.4,5 Ultimately, K-Ras is a negative predictive factor and potentially prognostic factor for CRC [6,7].

Recently, the surge in use of minimally invasive surgical techniques, such as laparoscopic and robotic surgery, has provided both short and long-term oncological results in CRC, sufficient for analysis. Surgical resection with en-bloc resection of affected organs and adequate lymphadenectomy are curative treatment modalities in resectable CRC patients [8]. Moreover, in local-advanced or metastatic CRC, a combination of surgery and systemic chemotherapy (SC) delivers a higher survival rate than surgery alone [9]. Recently, in metastatic CRC tumors, the addition of an anti-angiogenic agent such as bevacizumab to chemotherapy protocols in first and second-line therapy has been seen to extend both overall survival and progression-free survival [10]. On the other hand, there is some concern about complications in wound-healing induced by bevacizumab [11,12].

In this present study, our primary aim is to evaluate the risk factors for morbidity in patients with mutant or wild type Kirsten rat sarcoma undergoing colorectal cancer surgery. Secondly, we plan to assess the long-term results from our clinic.

Materials and Methods

Patients and Ethics

This study was conducted in the departments of surgery and oncology at our hospital between August 2010 and 2018. A total of 116 patients, who had been treated for colorectal cancer with or without metastasis were eligible for the study. KRAS mutation on surgically resected or biopsied specimens taken from patients with distant metastasis were analyzed. All cases were retrospectively evaluated which was collected data prospectively in terms of gender and age, BMI (body mass index), co-morbidity, ASA, mechanical intestinal obstruction, synchronous tumor, location of synchronous tumor, metastasis, recurrence, neoadjuvant chemotherapy and alterations in the biochemical parameter values from initial diagnosis to surgery; as well as operation time, surgical management, postoperative length of hospital stay, adjuvant chemotherapy, radiotherapy, monoclonal antibody treatment, tumor stage and grade at presentation; and follow-up, postoperative complications, complication classification and long-term survival. All patients underwent complete resection surgery where all identifiable disease was resected with curative intent. The survival rate was measured starting from the date of surgery with curative intent or date of presentation with unresectable metastatic disease, until the date of the last follow-up. As tumors in patients with synchronous metastatic disease were resected with curative intent, the survival time was calculated starting from date of the final resection. All patients were assessed in the preoperative period by an interdisciplinary oncology council. The study protocol was approved by the Ethical Committee (dated 5/10/2019 with Ethics Committee approval no. 715224473/050.01.04/4).

Chemotherapy and Radiotherapy

With regard to biological agents, all patients received treatment according to their RAS mutation status. The RAS wild-type tumors were treated with a combination of Cetuximab/Panitumumab combined with Oxaliplatin

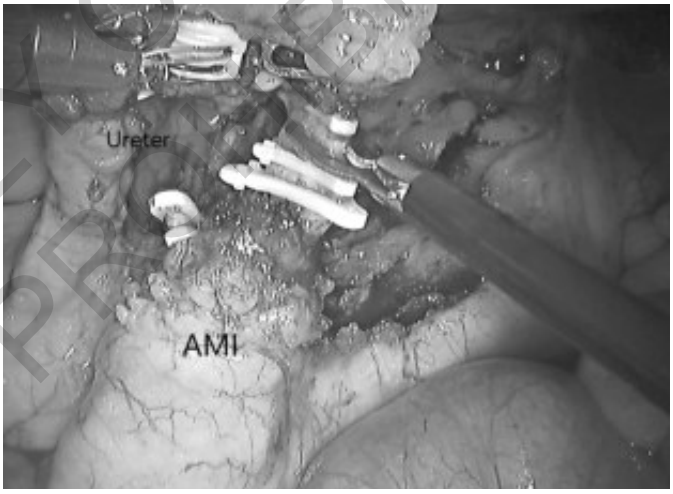

Fig. 1: Arteria mesenterica inferior and ureter during robotic low anterior resection (AMI: Arteria mesenterica inferior).

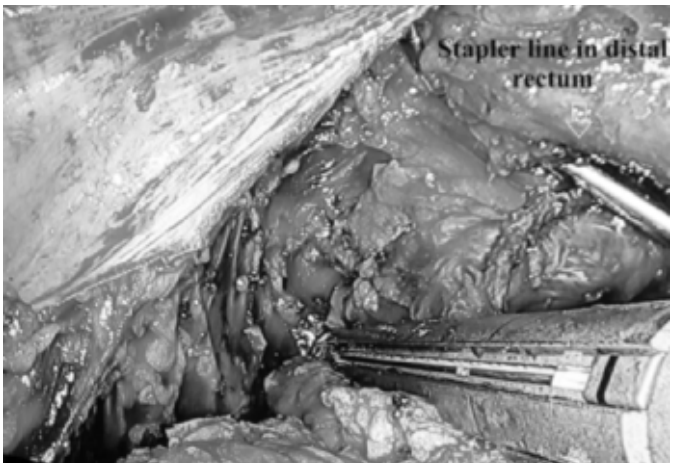

Fig. 2: Association of the stapler line and distal rectum in laparoscopic low anterior resection of mid-distal rectum cancer.

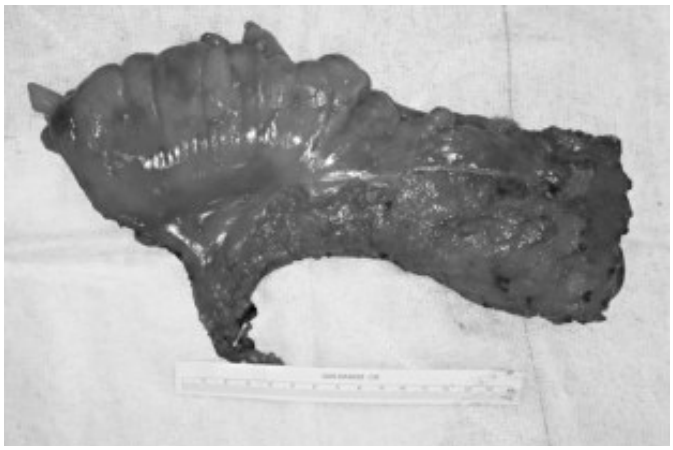

Fig. 3: Low anterior resection specimen after laparoscopic surgery.

or Irinotecan, a treatment based on a dual regimen (eg: Folfox/Capeox or Folfiri based). If the Oxaliplatin-based regimen was used as a first line treatment, this was switched to Irinotecan for the second-line treatment choice and vice versa. In the case of disease progression after using anti-EGFR agents, Bevacizumab was administered as a second-line combination.
Cetuximab/Panitumumab was replaced by Bevacizumab in patients with a mutant RAS-status. No patients were given palliative radiation in this study.

Methodology

A total of 116 patients with *wild/mutant*-KRAS who had been treated for colorectal cancer were enrolled in this study. All patients had undergone surgical treatment and were evaluated retrospectively. They were divided into two groups:
- Group 1: K(N)RAS wild type (n = 56),
- Group 2: K(N)RAS mutant type (n = 60)

The two groups were compared statistically in terms of gender and age, BMI and co-morbidity; preoperative and postoperative findings; morbidity and survival rates. The risk factors associated with survival were also identified. After being discharged from the hospital, the patient follow-ups were conducted at 1 week, 1 month and 6 months intervals.

Inclusion and Exclusion Criteria

Inclusion criteria for the present study comprised patients over 18 years of age, diagnosed with colorectal cancer with or without metastasis, undergoing colorectal cancer surgery, and with normal hemodynamic parameters (PTZ/INR, aPTZ, etc.). On the other hand, patients younger than 18 years of age, patients with undetermined KRAS status and patients either not undergoing surgical treatment or having surgery at a different hospital, were excluded.

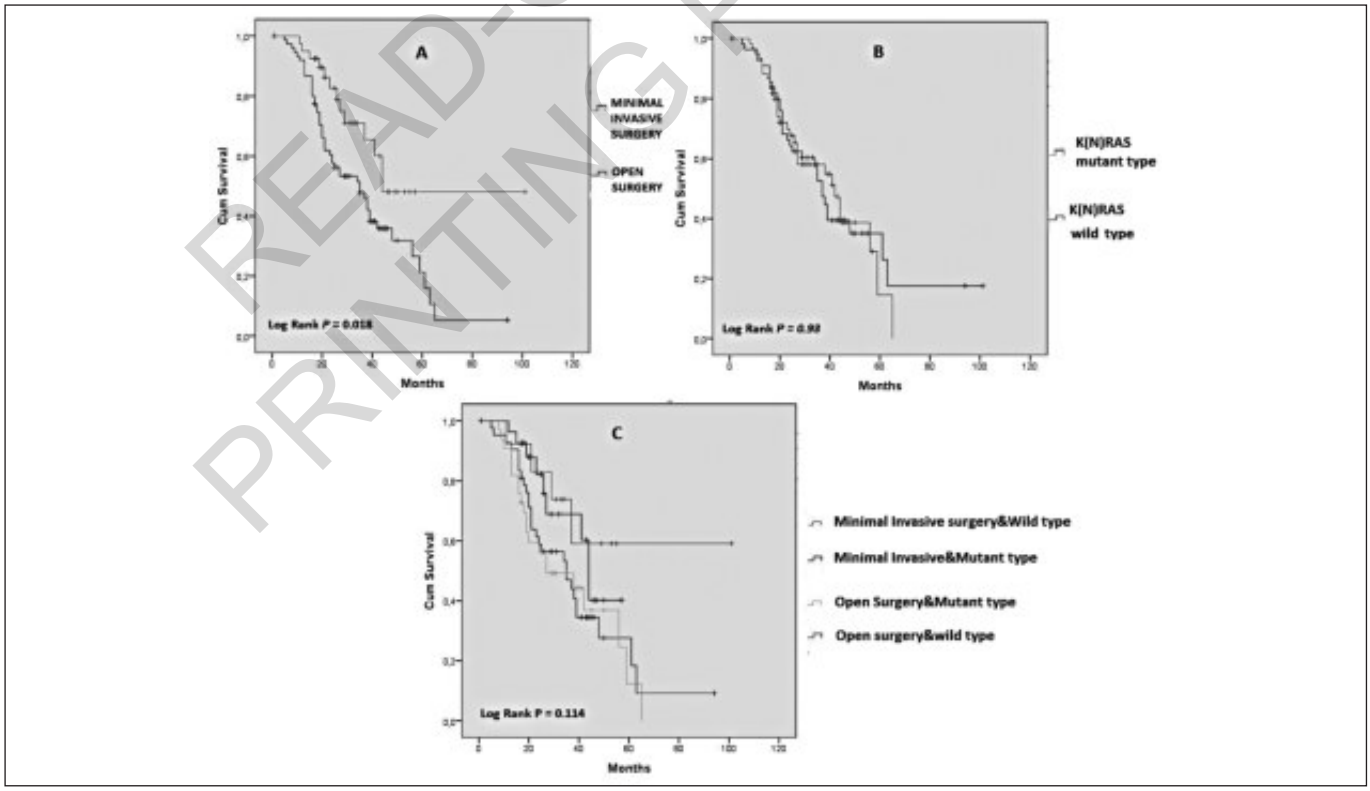

Fig. 4: Kaplan-Meier for survival rates in minimal invasive surgery (A), K(N)RAS mutant patients (B) and all groups (C).

Kras or Nras Mutation Analysis

In the present study, routine investigation for K(N)RAS mutation was carried out on patients who developed metastasis during follow-up or had synchronous tumor at the first diagnosis. KRAS mutations are often detected in codon 61, located in exon 2, as well as codon 12 (80%) and 13 (15%), both located in exon 1. The most common mutations in patients are Gly12Ala, Gly12Asp, Gly12Arg, Gly12Cys, Gly12Ser, Gly12Val, Gly13Asp. NRAS mutations are also frequently observed in the codon 61 region [5]. K(N)RAS mutation analysis was performed as described below using a hospital - approved procurement service provider. Using DNA material obtained from patient samples, replication was performed with a nested polymerase chain reaction (PCR) (2-step) method using codon-specific primers in which any mutations on KRAS and NRAS genes (1, 2 and 3 exons) could be detected. Later, these products were differentiated using fluorescently labeled ddNTPs to enable location of the target mutation sites using a mini-sequencing technique. Afterwards, the capillary electrophoresis technique was applied to complete the mutation analysis. The results obtained were then compared *with* the genome sequence of a normal individual and the mutation table of the gene from the GenBank Database. The examined exons were as follows: codon 12, 13, 59, 61, 117 and 146 for KRAS; codon 12, 13, 59, 61 and 146 for NRAS mutations.

Minimally Invasive Surgery Management

Laparoscopic surgery

Following general anesthesia, all patients were positioned in a Trendelenburg modified lithotomy position, with the right or left side facing down according to the side of the tumor. Prior to inserting the trocars, a Foley catheter was inserted into the bladder. A four or five-port technique was used on all patients and laparoscopic or robotic surgery was performed by different surgeons. The medial-to-lateral approach was adopted for all patients. A high-ligation of the inferior mesenteric artery or the ileocolic artery was usually performed for tension-free anastomosis, but in some cases a low-ligation of IMA was performed to preserve the left colic artery. In cases of sigmoid colon or rectum cancer, the splenic flexure and left colon were mobilized to achieve a tension free anastomosis. A Total Meso-rectal Excision (TME) was performed on all patients with mid and distal rectal cancers.

An extracorporeal or intracorporeal anastomosis was usually performed according to the surgeon's preference, the location of the cancer, and the course of operation, while in some cases a double-stapler anastomosis was preferred. The tumoral specimen was extracted through the upper part of the symphysis pubis or the umbilicus, on left and right-sided tumors, respectively. A loop ileostomy was performed on patients with neoadjuvant chemotherapy, prolonged operations or abnormal bleeding in rectum cancer. Hartmann's colostomy or ileostomy was successfully implemented on patients with a mechanical obstruction, with symptoms resulting from an anastomosis breakdown or with an enterocutaneous fistula). We used the subcutaneous drain in cases where there was especially fatty subcutaneous tissue. These drains form negative pressure which prevents an accumulation of fluid and leads to a reduction in dead space in the subcutaneous tissue.

Robotic-assisted surgery

Under general anesthesia, the patients were positioned in a Trendelenburg modified lithotomy position due to the tumor's location. A Foley catheter was inserted into the bladder before inserting the trocars. The robotic-assisted surgery was usually performed as a hybrid approach together with laparoscopy. A CO2 pneumoperitoneum of 12 mmHg was established using a Veress needle. A supra-umbilical port for the camera, three 8-mm robotic ports, and one assistant-laparoscopic trocar were inserted under direct observation in a semi-circular line linking both anterior and superior iliac spines and the umbilicus. Left colon and splenic flexure mobilization was generally completed laparoscopically, followed by a robotic arm grappling and docking.

The dissection and resection were performed similarly to laparoscopic surgery. After removing the specimen, the anastomosis was performed routinely as intracorporeal, on sigmoid and rectum cancer patients, with a circular-stapler.

On patients with right-sided cancer, the anastomosis was removed using the double-layer linear stapler or the handsewn-anastomosis technique. In fact, the preferred anastomosis technique may change according to the surgeon's experience.

Open surgery

Open surgery was more commonly preferred in cases of symptomatic patients, those with a mechanical obstruction or where adhesion to other organs or structures led to the CRC. Following general anesthesia, patients were positioned in a Trendelenburg modified lithotomy position for left-sided cancers or supine position for right-sided cancers. After entering the abdomen, checks were made for distant metastasis. In most patients, we chose a dissection using the lateral-to-medial method. This dissection was achieved in a similar way to the minimally invasive method.

Postoperative Care, Anticoagulation and Follow-Up

Intensive monitoring of pulse, blood pressure, temperature and drains on all patients was provided in the early postoperative hours. The hemoglobin, platelet count and International Normalized Ratio (INR) rates were checked after surgery. Patients with high ASA scores were taken into the intensive care unit. Oral feeding was started after gastrointestinal functions had been established. The drain was removed when the output was less than 30 ml over 48h. Most of the patients were discharged with low-molecular weight heparin (LMWH) at 0.4/0.6 IU/mL during the first 3 weeks of the postoperative period. The patients were followed closely during the first month to ensure healthy wound healing and hemodynamic parameters. After this period, further follow-up was carried out by the Oncology department clinic.

Statistical Analysis

Statistical analysis was evaluated using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). The descriptive statistical methods were used to assess the present study. The number of patients and percentage (%) were used for categorical data. There were two independent groups in study. The categorical variables were tested by Pearson χ^2 and Fisher's exact test to compare qualitative date in present study. The normality of data distribution was determined by the Kolmogorov-Smirnov test. While Student's t test was used to compare for mean differences in the normally distributed data, Mann Whitney U test was performed for comparisons of the not normally distributed data. Determining the potential factor associated with surgical site infection in the K(N)RAS wild/mutant patients, the multivariate logistic regression analysis was used. Overall survival was compared was using the Kaplan-Meier method, and the comparisons were performed using the log-rank test. Determining the best factor (s) associated with survival in the K(N)RAS wild/mutant patients underwent open or minimal invasive surgery, variables were determined using univariate analysis. Any variables whose univariable test had a *P* value <0.05 was accepted as a candidate for a Cox proportional hazards multivariable model along with all variables of known clinical importance. Based on the results, a P value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Patient Characteristics and Frequency of K(N)Ras Mutations

Out of a total of 130 patients, 116 were enrolled in the study, with 14 patients being excluded (undetermined mutation type in four cases, inadequate clinical information in four cases, operation or follow-up in different hospital in three cases, out of study period in two cases) for failing to meet the inclusion criteria. A total of 47 (40.5%) female patients and 69 (50.5%) male patients were included in the study (*P* = 0.62). The median age for the K(N)RAS wild type group was 60 (44-83) years and 68.5 (24-81) years for the KRAS mutant group (*P* = 0.0019). There was no significant difference in terms of body mass index, co-morbidities, with the most common being anemia (41.4%), hypertension (37.9%), diabetes mellitus (27.6%) and patients with more than three co-morbidities (44.7%) (P = 0.233). The median ASA score for all patients was 2 (1-4), and of the 45 (38.7%) had multi-comorbidity (*P*> 0.05) (Table I).

The K(N)RAS mutation status was checked routinely in patients who developed metastasis during follow-up or had synchronous tumor at initial diagnosis. Of the 116 patients who underwent surgery, KRAS mutation was found in 51 cases (43.9%) and 9 cases (7.7%) had NRAS mutation. The majority of KRAS mutations (25 patients, 21.5%) were in exon 2 codon 12, while NRAS mutations were mostly in exon 2 codon 61 (6 patients, 5.1%). The median preoperative Carcino Embryogenic Antigen (CEA) rate was 6.1 (1-1500) μg/L (*P* > 0.05). Most tumors were located on the left side (Right vs Left side: 26% vs 74%) (P =0.409). Of the 116 patients, 50 (43.1) had synchronous tumors (*P* = 0.002), most of which were located in the liver (70%) (*P* = 0.085). The rate of tumor recurrence was 3 (2.8%). The number of symptomatic patients or patients with mechanical bowel obstruction was 31 cases (26.7%) (*P* = 0.213) in groups. The rate of conversion to open surgery was 7.7%. The number of minimal invasive surgery in group 1 was higher than in group 2 (P = 0.014) (Table 1).

No significant difference was found in terms of the median length of hospital stay (8.5 (3-74) days), types of treatment such as metastasectomy or ablation (31.8% in all patients), the median length of the resected specimen (23 cm (8-82)), the median diameter of the resected tumor (4.5 cm (1-15)), the median number of removed lymph nodes (14 (3-53)), or the median follow-up duration (27 months (1-101)) between the groups (*P*>0.05). The median operation time was higher in the mut-K(N)RAS groups than in the wild- K(N)RAS groups (*P*=0.035) (Table I).

Morbidity

The rate of superficial surgical site infection was 25.8%; and was more common in both the open surgery K(N)RAS groups (P=0.001); these patients received wound care. Patients with pleural effusion or atelectasis (12.1%) were assigned a bronco-dilatator, acetylcysteine

Table I - *Patient's characteristics*

	Total (n=116)	KRAS Wild type (n =56)	KRAS Mutant type (n = 60)	P value
Age	62 (24-84)	60 (44-83)	68.5 (24-81)	0.019
Gender (M/F) (%)	69 (59.5)/47(40.5)	24 (42.8)/32 (57.2)	23(38.3)/37 (61.6111)	0.62
Body mass index (kg/m2)	22 (17-38)	22 (19-38)	21.5 (19-30)	0.363
Comorbidity (≥3)	51 (43.9)	27 (48.2)	24 (40)	0.233
Smoker	25 (21.5)	15 (26.7)	10 (16.6)	0.4
ASA (≥3)	45 (38.7)	20 (35.7)	25 (41.6)	0.511
CEA (>5)	62 (53.4)	29 (51.7)	33 (55)	0.138
Location (Right / Left side)	31(26.7)/ 85(73.3)	13(23.2)/43(76.7)	18 (30)/42 (70)	0.409
Sencronus tumor	50 (43.1)	23 (41)	27 (45)	0.78
Recurrent Tumor	3 (2.5)	1 (1.7)	2 (3.3)	>0.999
Symptomatic or MBO	31 (26.7)	12 (21.4)	19 (31.6)	0.213
Conversion to open surgery	9 (7.7)	5 (8.9)	4 (6.6)	0.737
Minimal invasive surgery	40 (34.4)	13 (23.2)	27 (45)	0.014
Iatrogenic perforation	12 (10.3)	9 (16)	3 (5)	0.109
Length of the hospital stay	8.5 (3-74)	11 (4-33)	8 (3-38)	0.272
Operation time (min)	210 (90-600)	180 (120-420)	215 (90-600)	0.035
Metastazectomy or ablation	37 (31.8)	21 (37.5)	16 (26.6)	0.211
Length of resected specimen (cm)	23 (8-82)	20 (11-65)	24.25 (12-70)	0.743
Tumor diameter (cm)	4.5 (1-15)	4.25 (1-15)	4.75 (2-12)	0.637
Number of the removed LN	14 (3-53)	15.5 (5-49)	13 (4-32)	0.08
Number of removed metastatic LN	2 (1-43)	2.5 (1-43)	2.5 (1-17)	0.497
Radial surgical positivity	11 (9.4)	6 (10.7)	5 (8.3)	0.903
Curative resection	105 (90.52)	50 (89.2)	55 (91.6)	0.789
Follow up (month)	27 (1-101)	29 (1-101)	25 (8-65)	0.31
Complication (with subgroup)		Open Surgery / MIS	Open Surgery / MIS	
sSSI	30 (25.8)	18 (32.1) /-	12 (20) / 2 (3.3)	0.001a
Pleural effusion, atelectasis	14 (23.3)	5 (8.9)/1 (1.7)	5 (8.3) / 3 (5)	0.932
Postoperative ileus	(11.2)	-/ 4 (7.1)	4 (6.6) / 5 (8.3)	0.382
Stoma related complication	11 (9.4)	3 (5.3)/1 (1.7)	3 (5)/ 4 (6.6)	0.834
Anastomosis leak	10 (8.6)	5 (8.9) /-	-/5 (8.3)	0.031b
Hemorrhage	7 (6)	5 (8.9) /-	-/ 2 (3.3)	0.139
Clavien&Dindo (≥3A)	19 (16.3)	6 (10.7) / -	7 (11.6) / 6 (10)	0.255

ASA: American Society of Anesthesiologists, CEA: Carcinoembryonic antigen,Clavien&Dindo: Clavien and Dindo complication classification, F/M:Female/Male, MBO: Mechanic bowel obstructive, MIS: Minimal invasive surgery, sSSI: Superficial surgical site infection, a (wild-KRAS of Open surgery vs MIS group; P=0,03), b (mut-KRAS groups vs wild-KRAS groups P<0,05)

and a series of exercises to increase pulmonary capacity following lung disease consultation.

Out of the 116 patients, 13 (11.5%) had mechanical bowel obstruction after the operation. Medical treatment comprising the cessation of oral feeding and initiation of parenteral hydration with or without nasogastric drainage was applied in 6 patients (5.2%). However, 7 of the 13 patients required surgery: 3 (2.6%) had a bridectomy, 2 (2.6%) had cyto-reductive surgery due to peritoneal carcinomatosis (PC) and 2 (2.6%) had ileostomy as a result of PC.

Stoma-related complications occurred in 11 (9.4%) patients. Out of those 11 patients, 9 were given stoma nursing care with washing using 0.9% NaCl and a local stoma revision at the bedside. The other 2 patients (2.6%) underwent an operation for stoma revision.

Anastomosis leakage developed in 10 (8.6%) patients. These patients underwent an operation to remove the anastomosis, where debridement was performed and an opening for an ostomy was created. Minor hemorrhaging occurred in 7(6%) of the patients and was brought under control by close monitoring of hematocrits, use of fresh-frozen plasma, Vitamin K and an erythrocyte suspension.

Out of the 116 patients, 19 had complications scoring equal to or higher than grade 3 according to Clavien-Dindo [14]. This classification of complications is defined as: grade 1 - involving minor risk events not requiring treatment; grade 2 - complications demanding pharmacological treatment with drugs other than those granted for grade 1 complications; grade 3 - complications leading to continuing impairment or organ resection; grade 4 - life-threatening complications, and according to this scale, the mut/wild-K(N)RAS group had the highest complication rate (≥3A) (P = 0.006) (Table I).

Histopathological Findings, Operations and Treatment Protocols

The medians of the clinical stage and the T score were 3 (2-4) and 3 (2-4) (P > 0.05, respectively). The status of the lymph nodes, metastasis, tumor implant or nodule, mucinous component, tumor grade, vascular, lymph node and nerve invasion were similar in all of the groups (P > 0.05). A statistically significant correlation was found in terms of adjuvant treatment protocols (Folfox/Folfiri or Xelox;

TABLE II - *Histopathological finding and treatment protocols in groups*

	Total (n=116)	KRAS Wild type (n =56)	KRAS Mutant type (n = 60)	P value
Clinical Stage				0.281
2	18 (15.5)	6 (10.7)	12 (20)	
3	53 (45.6)	29 (51.7)	24 (40)	
4	45 (38.7)	21 (37.5)	24 (40)	
TNM staging				
T				0.114
2	6 (5.1)	5 (8.9)	1 (1.6)	
3	53 (45.6)	27/ (48.2)	26 (43.3)	
4	56 (48.2)	23 (41)	33 (55)	
N positivity	81 (69.8)	42 (75)	39 (65)	0.273
N >1	39 (33.6)	20 (35.7)	19 (31.6)	0.845
M	45 (38.8)	21 (37.5)	24 (40)	0.782
Tumor implant or nodule	17 (14.6)	7 (12.5)	10 (16.6)	0.52
Mucinous component	19 (16.4)	8 (14.2)	11 (18.3)	0.556
Tumor grade				0.977
Well	29 (25)	10 (16.6)	15 (25)	
Moderate	55 (47.4)	27 (48.2)	28 (46.6)	
Poor	14 (12)	6 (10.7)	8 (13.3)	
Vascular invasion	78 (67.2)	41 (73.2)	37 (61.6)	0.239
Lymphatic invasion	80 (68.9)	43 (76.7)	37 (61.6)	0.218
Nerve invasion	47 (40.5)	19 (33.9)	28 (46.6)	0.376
Treatment protocol				
Neoadjuvant treatment	36 (31)	16 (28.5)	20 (33.3)	0.624
Bevacizumap*	6 (5.2)	3 (5.3)	3 (5)	0.921
Cetuximap*	1 (0.8)	1(1.7)		
Adjuvant treatment				
Folfox/Folfiri or Xelox	100 (86.2)	52 (92.8)	48 (80)	0.044
Bevacizumap or Aflibercept	55 (47.4)	25 (44.6)	30 (50)	0.392
Cetuximap	19 (16.3)	17 (30.3)	2 (3.3)	<0.001
Panitumumap	11 (9.4)	11 (19.6)	<0.001	<0.001
RT (long-period)	45 (38.7)	18 (32.1)	27 (45)	0.381

ASA: American Society of Anesthesiologists, CEA: Carcinoembryonic antigen, F/M: Female/Male, LN: Lymph node, MIC: Minimal invasive surgery, RT: Radiotherapy, SSI: Superficial surgical infection, TNM: TNM Classification of Malignant Tumors, *: given in addition to conventional Ct

Cetuximap and Panitumumap*)* (P = 0.044, P < 0.001, respectively). On the other hand, no significant difference was found between conventional neoadjuvant treatments (P > 0.05). The numbers of patients given radiotherapy (long-duration) were similar in all the groups (*P* = 0.381) (Table II).

Considering the cohort of 116 patients, the following colorectal procedures were the most frequently performed: low anterior resection (43.9%) (25% open surgery, 16.3% laparoscopy, 2.5% robotic surgery); liver metastasectomy (+/- ablation or TACE) 37% (31.8%); right hemicolectomy (25%) (21.5% open surgery, 2.5% laparoscopy, 0.8 robotic surgery), left hemicolectomy (9.4%) (6% open surgery, 1.7% laparoscopy, 1.7 robotic surgery). (Table III).

Surgical Site Infection (SSI)

In the present study, the term 'superficial surgical site infection" includes superficial incisional and deep incisional infections seen either together or independently.

The effects of different treatment protocols on superficial surgical site infection rates were assessed according to the groups and the highest rate was seen in patients given Bevacizumab during the neoadjuvant treatment (OR=140.339) (4.303-4581.307) (P=0.005).

Additionally, the SSI rate was at its lowest in cases where minimally invasive surgery had been performed, and this rate fell up to 16.1 times as the duration of MIS was reduced (OR=0.062) (0.006-0.628) (P=0.019) (Table IV). Furthermore, when a complication of grade 3 or higher on the Clavien-Dindo scale occurred, it was significantly associated with an increased SSI rate with a 9 times greater risk (95% CI= 1.817-50.953) (P= 0.008). We also analyzed and assessed organ and space infections, but no statistically significant result was detected.

Overall Survival

After a median 27 months (min-max:1-101), 62 (53.4%) patients out of 116 had died. The overall median survival rate for all patients was 38±3.1 (31-44) months, with a cumulative proportion of 1, 3 and 5-year survival rates of 93%, 52.2% and 21.5%, respectively. The

Table III - *Operation procedures*

	Total(n,%)	Open surgery (n =76)	Minimal invasive surgery (n =40) Laparoscopic surgery	Robotic surgery
Low anterior resection	51 (43.9)	29 (38,1)	19 (47,5)	3 (7,5)
Liver metastazectomy, Ablation or TACE	37 (31.8)	30 (39,4)	7 (17,5)*	
Right hemicolectomy (± extended)	29 (25)	25 (32,8)	3 (7,5)	1 (2,5)
Sigmoid or anterior resection	12 (10.3)	10 (13,1)	2 (5)	
Left hemicolectomy (± extended)	11 (9.4)	7 (9,2)	2 (5)	2 (5)
Miles procedure	8 (6.8)	5 (6,5)	2 (5)	1 (2,5)
Colectomy with Splenectomy or distal pancreatectomy	6 (5.1)	6 (7,8)		
Subtotal/total colectomy	4 (3.4)	4 (5,2)		
Low anterior resection + TAH&BSO	2 (1.7)	2 (2,6)		
Colectomy + Whipple procedure	1 (0.8)	1 (1,3)		
Right hepatectomy	1 (0.8)	1 (1,3)		
Lung metastazectomy	2 (1.7)	2 (2,6)		
Cholecystectomy	2 (1.7)	2 (2,6)		
Pelvic lymphadenectomy	2 (1.7)	1 (1,3)	1 (2,5)	
Others				
Liver or peritoneal biopsy	8 (6.8)	1 (1,3)	3 (7,5)	
Cystoscopy or ureter catheter	4 (3.4)			

TACE: Trans-arterial chemoembolization, TAH&BSO: Total Abdominal hysterectomy and bilateral Salpingo-Oophorectomy, * Ablation or TACE

Table IV - *Assessment of the risk factors on superficial surgical site infection with multivariate logistic regression analysis*

	B	OR	95% CI	Wald	P
Minimal invasive surgery	-2.786	0.062	0.006-0.628	5.538	0.019
Neoadjuvant Bevacizumab treatment	4.944	140.339	4.303-4581.307	7.732	0.005
Co morbidity (≥3)	-0.018	1.0183	0.234-4.116	0.01	0.98
BMI	0.055	1010	0.0103-39.136	0.213	0.639
Age	-0.032	1.031	0.916-1.025	1.223	0.269
ASA (≥3)	1.316	3.728	0.903-15.396	3.308	0.069
Smoker	1.056	2.874	0.649-12.724	1.934	0.169
MBO or symptomatic patients	-0.446	1.562	0.640-2.872	0.339	0.561
Clavien&Dindo complication (≥3)	2.264	9.623	1.817-50.953	7.089	0.008

ASA: American Society of Anesthesiologists, BMI: Body mass Index, MBO: Mechanic bowel obstruction, OR: Odds ratio, CI: Confidence interval

overall median rate of survival of the MIS group (44 months) was longer than the open surgery groups (35±5.5 months (24.1-45.8)) (P = 0.018). The overall mean/median survival rate of K(N)RAS wild and mutant types was 45.4±5.4(34.8-56) / 37±2.2(32.5-41.4) and 39.3±2.9 (33.4-45.1) / 42±7(28.1-55.8) months, respectively. Considering K(N)RAS wild (mean for survival time) and mutant patients separately, their average survival rates for MIS were 70±12.2 (46.6-94.5) and 44±2.1(39.8-48.1); and for open surgery were 35±7.7 (19.7-50.2) and 27 (6.2-47.7), respectively (P = 0.114). One patient (0.8%) died during surgical hospitalization (hospital mortality) due to sepsis from fecal peritonitis resulting from a colon tumor perforation.

Univariate analysis revealed many factors to be predictive of overall survival rate: CEA (above 5 μg/L), MIS, tumor location, tumor stage, TN staging, curative resection, length of removed specimen, tumor diameter, metastasectomy or ablation, number of removed metastatic lymph nodes, tumor grade, vascular and lymphatic invasion and the presence of tumor implant or nodule (Table V). Multivariate analysis showed that CEA (>5ng/mL) (HR 2.94 (95% CI 1.337-6.492)) (P=0.007); tumor stage (Stage 2, (P=0.034)), T staging (T2) (P<0.001), T4 stage (HR 1.91 (95% Cl 1.605-252.6) (P=0.02); metastasectomy with(out) ablation (HR 0.19 (95% 0.077-0.520) (P=0.001); the number of removed metastatic lymph nodes (HR 1.08 (1.010-1.155) (P=0.025); and tumor implant or nodule (HR 2.71 (1.102-6.706) (P=0.03) to be factors affecting the overall survival rate (Table V).

Discussion

CRC is an important health problem throughout the world and can cause many complications including obstruction, invasion, perforation and metastasis if not

TABLE V - *Univariate and multivariate statistical analysis of overall survival stratified by clinical and pathological features*

	Hazard Ratio (95% Confidential Interval)			
	Univariate	P-value	Multivariate	P-value
Gender	1.008 (0.603-1.684)	0.977		
Age>65	1.146 (0.674-1.949)	0.682		
Comorbidity (>3)	1.015 (0.867-1.666)	0.956		
ASA>2	1.162 (0.682-1.980)	0.581		
CEA>5	2.08 (1.208-3.611)	0.008	2.942(1.337-6.492)	0.007
Tumor location	0.532 (0.313-0.906)	0.02	0.743(0.328-1.709)	0.492
Synchronous tumors	1.323 (0.795-2.190)	0.283		
Emergency operation	1.49(0.839-2.656)	0.173		
K(N)RAS mutation status	0.971 (0.592-1.615)	0.931		
Minimal invasive surgery	0.482 (0.264-0.899)	0.022	0.719 (0.356-1.796)	0.588
Length of the hospital stay	1.065 (0.991-1.141)	0.086		
Clavien&Dindo complication (≥3A)	1.52 (0.828-1.591)	0.177		
Tumor stage				
2	1 (reference)	0.085		0.034
3	2.291 (0.951-5.529)	0.064		
4	2.743 (1.125-6.677)	0.026	3.235(0.727-14.374)	0.123
TNM staging				
T 2	1(Reference)	<0.001		<0.001
3	1.241 (0.285-5.401)	0.773		
4	5.102 (1.221-21-344)	0.026	1.917 (1.605-252.6)	0.02
N	1.951 (1.053-3.625)	0.034	0.538 (0.157-1.81)	0.314
M	1.453 (0.875-2.406)	0.149		
Curative resection	0.481 (0.256-0.909)	0.014	2.40 (0.878-6.580)	0.042
Length of resected specimen	1.027(1.009-1.045)	0.003	0.99 (0.966-1.025)	0.731
Tumor diameter	1.18(1.068-1.312)	0.001	1.066 (0.890-1.275)	0.488
Metastasectomy or ablation	0.461 (0.254-0.841)	0.012	0.198 (0.077-0.520)	0.001
Iatrogenic perforation	1.258 (0.589-2.666)	0.557		
Number of the removed LN	1.029(0.999-1.056)	0.056		
Number of the metastatic removed LN	1.075 (1.039-1.119)	<0.001	1.089 (1.010-1.155)	0.025
Grade				
Well	1 (Reference)	0.026		0.258
Moderate	1.635 (0.802-3.330)	0.177		
Poor	2.211 (0.981-4.984)	0.004	1.549 (0.465-5.130)	0.924
Vascular invasion	2.452(1.268-4.749)	0.008	2.597 (1.470-4.561	0.924
Lymphatic invasion	2.873 (1.356-6.096)	0.006	<0.001(0.0001-3.257)	0.925
Nerve invasion	1.682(0.924-3.084)	0.089		
Mucinous component	1.64 (0.905-3001)	0.102		
Tumor implant or nodule	4.917 (2.676-9.021)	<0.001	2.714 (1.102-6.706)	0.03
Neoadjuvant treatment	0.027 (4.593-1587366.5)	0.692		
Adjuvant treatment				
Xelox	0.571 (0.106-3.167)	0.527		
Folfox or Folfiri	0.785 (0.230-2.692)	0.703		
Xelox and Folfox or Folfiri	1.158 (0.405-3.267)	0.792		
Other KT or NA	0.842(0.281-2.530)	0.760		
Monoclonal antibody with Ct	1.35 (0.741-2.474)	0.324		
Cetuximab or Panitumumab	0.75(0.418-1.359)	0.348		
Bevacizumab or Aflibercept	1.319 (0.766-2.248	0.322		

ASA: American Society of Anesthesiologists, CEA: Carcinoembryonic antigen, Ct: Chemotherapy, F/M: Female/Male, MIC: Minimal invasive surgery, A: Not available RT: Radiotherapy, SSI: Superficial surgical infection, TNM: TNM Classification of Malignant Tumors

treated effectively. The number of annual incidences in female and male patients is 34.3 and 45.2/100.000, respectively, with more than 27,642 related deaths occurring in 2016 in USA.1 CRC is a heterogeneous disease, in whose tumorigenesis genomic and epigenetic alterations frequently play an important role [2,3]. The adenoma-carcinoma model in the Fearon and Vogelstein report is based mainly on the tumorigenesis of CRC. KRAS mutation plays an important role in tumorigenesis, and it has been reported as a negative predictive factor and/or as a potential factor for a poor prognosis [4]. So, in patients with KRAS mutations, it has been recommended that surgical and medical treatment should be performed in an interdisciplinary and meticulous manner as in cases with local-advanced tumor and metastasis, colorectal cancer can follow an aggressive course with poor prognosis [5-7]. The only curative treatment for CRC is the complete surgical resection of local tumors and of cer-

tain advanced local tumors that are operable [8-10]. Moreover, with some selected patients with distant metastases of CRC, including in their liver and lungs, if the resection of the primary tumor can be carried out along with metastasectomy, this can lead to a progression-free disease [11-13]. A colectomy or low-anterior resection is recommended for "resectable" tumors, and in these cases the surgeon can achieve successful resection through open surgery, laparoscopic surgery or robotic surgery. MIS is a feasible and safe technique and growing in popularity for application in colorectal cancer as well as in other oncological cancers [14]. Moreover, laparoscopic and robotic surgical treatments for colorectal cancer are performed with increasing frequency in our current surgical clinic, in parallel with increasing technological developments and surgical experience. These methods are favored due to low postoperative pain, decreased use of analgesic and lower hospital stay rates. A meta-analysis of oncological results in 10 randomized-controlled trials with 3,830 patients conducted by Jackson et al. [14] did not show any significant difference in terms of the oncological end points between laparoscopic surgery and open surgery. Moreover, it was suggested that laparoscopic surgery seemed to be a good surgical option for colorectal surgery [14]. In a randomized clinical trial (COLOR study) 15 of 1,248 patients with colon cancer undergoing open surgery or laparoscopic surgery, a comparison of surgical methods reported a non-significant difference in disease-free survival after 3 years, with a difference of 2.0% favoring the open colectomy. Furthermore, this study was unable to show the non-inferiority of the laparoscopic surgery, as the upper limit of 95% CI for the difference exceeded the predetermined non-inferiority boundary of 7% [15]. A meta-analysis (24 studies with 4,592 patients in the laparoscopic surgery group and 3,865 patients in the open surgery group) of laparoscopy versus open surgery in the treatment of CRC showed that laparoscopic surgery is superior due to a significant decrease in estimated blood loss, shorter hospital stays, postoperative mortality and presence of postoperative complications [15,16]. In the present study, of the 116 patients, 40 cases (34.4%) underwent laparoscopic or robotic surgery. The rate of conversion to open surgery was 7.7% (9/116). Moreover, the median length of hospital stay (8.5 (3-74 days), operation time (210 (90-600) minutes), length of resected specimen (23 (8-82) cm), number of lymph nodes removed (14 (3-53)), and curative resection rate (90.52%) were similar to the literature [15,16].

Robotic-assisted surgery is a preferred surgical procedure and has become the most popular minimally invasive technique in various clinical practices [15-17]. A meta-analysis of CRC treatment comparing robot-assisted colorectal surgery (RACS), laparoscopic-assisted colorectal surgery (LACS), and open surgery carried out by Sheng et al. [17] suggested that RACS could be a better treatment for patients with CRC. Zhang et al. [18], in a meta-analysis evaluating patients undergoing colorectal surgery with either robot-assisted or laparoscopic-assisted surgery, also reported RACS to be a promising surgical approach because of its safety and efficiency compared to the LACS procedure. However, the study advised that further research was necessary to analyze the long-term cost-efficiency, functional and oncological outcomes of RACS. In the present study, robotic surgery was used in 7 cases, and only one patient (2.5%) undergoing MIS required conversion to open surgery.

Wound infection or surgical site infection can occur after colorectal surgery. The SSI rate varies between 2%–9% for MIS versus 3%–30% for open surgery in CRC.20,21 Yamamoto et al20 and other experienced colorectal surgeons have reported a lower SSI rate in MIS cases compared to those having open surgery. There are several risk factors for SSI including surgical technique, hemostasis, sterile field breeches, presence of foreign bodies, extended operative time, experience of the surgeon, intraoperative heart pressure, stoma creation, antibiotic prophylaxis, advanced age of patients (over 75 years), diabetes mellitus, severe cardiological diseases, advanced cancer, and emergency surgery [19-22]. In our clinic, we strive to reduce the effects of the above-mentioned factors and, in addition, we use often the laparoscopy or robotic surgery in suitable CRC cases. In cases with particularly fatty subcutaneous tissue subcutaneous drains are used. As these drains provide negative pressure, fluid cannot accumulate; thus, dead space is reduced in the subcutaneous tissue. In the present study, the superficial surgical infection rate was 25.8%.

This sSSI rate was noticed to be at its lowest in cases undergoing MIS, while the rate decreased 16.1 times as the duration of MIS was reduced (OR=0.062) (95% CI= 0.006-0.628) (*P*=0.019).

Bevacizumab is a monoclonal antibody, which affects the vascular endothelial growth factor (VEGF) and is administered in combination with conventional chemotherapy in colorectal cancer.23 As these anti-angiogenic agents affect the neovascularization process of normal wound repair as well as tumor growth, there is a concern about the impairment or delay in wound healing [23-25].

Bevacizumab, as an anti-angiogenic agent, has a long terminal half-life, predicted to be 20 days (range:11-50); therefore, its effects may continue throughout any interruption of the drug treatment during the perioperative period [25,26]. In this present study, patients receiving *Bevacizumab* as neoadjuvant treatment had a significantly higher sSSI rate; whereas, in patients undergoing minimally invasive surgery, this rate was significantly lower. Patients given *Bevacizumab* as neoadjuvant treatment had the highest superficial sSSI rate. Moreover, the SSI rate was highest in cases where *Bevacizumab* was given as neoadjuvant therapy, and this rate increased 140 times as the *Bevacizumab* treatment was increased (OR=140.339) (95% CI= 4.303-4581.307) (P=0.005). Therefore, we suggest that in patients who have received

Bevacizumab as neoadjuvant treatment, surgery should be delayed by at least eight weeks.

Anastomotic leak (AL) is one of the most dreaded complications after CRC surgery. Re-operation can be necessary in patients with an uncontrollable leakage or sepsis. The rate of AL varies from 3% to 12% in the literature.27 Risk factors affecting AL can be the location of the tumor; the surgical approach (open/laparoscopy/robotic); patient characteristics such as gender; co-morbidities, especially diabetes; and patient lifestyle [28,29]. AL or intestinal fistula can cause severe morbidity, and a delay or failure in delivering chemoradiotherapy may result in tumor recurrence or metastasis.30 Moreover, AL is frequently associated with grade 3 complications on the Clavien-Dindo scale. In the present study, 19 complications of Grade 3 or higher were recorded; of these, AL was the most common, seen in 9 cases (8.6%), while uncontrolled fistula occurred in 4 cases (3.4%). This rate of AL was similar to the literature. Also, in cases with complications rating Grade 3 or higher on the Clavien-Dindo scale, there was a significant association with an increased rate of SSI, with a 9 times greater risk (B= 2.264) (OR= 9.623) (95% CI= 1.817-50.953) (B= 0.008).

In the present study, the overall median length of survival was 38 (31-44) months, while the overall median rate of survival of the MIS group was longer than the open surgery groups (44 versus 35 months) (P = 0.018). We think this result may be due to the fact that open surgery was carried out on patients with significantly advanced stages compared to the minimally invasive group, especially in terms of clinical staging, T (primary tumor) and metastasis. In our study, we found the overall survival rate was affected by these factors: CEA (>5ng/mL) (HR 2.94 (95% CI 1.337-6.492)) (P=0.007); tumor stage (P<0.05), T staging (P<0.05), metastasectomy or ablation (HR 0.19 (95% 0.077-0.520) (P=0.001); the number of removed metastatic lymph nodes (HR 1.08 (1.010-1.155) (P=0.025); and tumor implant or nodule (HR 2.71 (1.102-6.706) (P=0.03).

There are some limitations to this present study. Firstly, the sample sizes were small and the study was planned retrospectively. Secondly, minimally invasive surgery did not emerge as an effective factor for survival rate in multivariate analysis. This result may stem from the small size of the samples in the groups. Thirdly, minimally invasive surgery was mostly performed during the second half of the study, and as the sample numbers were small, the probability of the cumulative proportion not surviving 5 years could not be assessed. Moreover, the open surgery groups had advanced clinical stage and TNM staging. On the other hand, the main outcome of our results was the significantly higher sSSI rate recorded in patients receiving Bevacizumab as neoadjuvant treatment. *Bevacizumab* is a monoclonal antibody which affects the vascular endothelial growth factor (VEGF) and is administered in combination with conventional chemotherapy in colorectal cancer. In patients with mutated KRAS or cases with progression after using anti-EGFR agents, Bevacizumab is used for second line therapy.

Conclusion

The use of *Bevacizumab* during neoadjuvant treatment, the choice of surgical approach and complications of Grade 3 or higher on the Clavien-Dindo scale are all risk factors for surgical site infection in patients with *mut/wild*-K(N)RAS undergoing colorectal cancer surgery. Furthermore, with respect to surgical site infections following neoadjuvant treatment in patients to be operated on for colorectal cancer, we recommend extreme care in the timing and use of anti-angiogenic agents. Moreover, CEA (>5ng/mL), tumor stage, T stage, metastasectomy or ablation, the number of removed metastatic lymph nodes, tumor implant or nodule and curative resection are all risk factors for the overall survival rate.

Riassunto

Contesto: nel cancro del colon-retto (CRC), la mutazione del gene K(N)RAS ha un impatto significativo sul decorso clinico ed è associata a una prognosi negativa. Il nostro obiettivo è presentare la morbilità e i risultati a lungo termine in pazienti con wild/mut-K(N)RAS, sottoposti a chirurgia CRC.

Metodi: In questo studio retrospettivo sono stati inclusi un totale di 116 pazienti sottoposti a intervento chirurgico per tumori del colon-retto con wild/mut-K(N)RAS. I pazienti sono stati divisi in due gruppi: pazienti con K(N)RAS non mutato (Gruppo 1) e pazienti con K(N)RAS mutato (Gruppo 2). I risultati sono stati valutati per gli esiti clinici, operativi, di morbilità e di sopravvivenza a lungo termine.

Materiali e metodi: Il più alto tasso di infezione del sito chirurgico (SSI) (OR=140,339) (4,303-4581,307) (P=0,005) è stato osservato nei pazienti trattati con Bevacizumab durante il trattamento neoadiuvante. Nel frattempo, il tasso di infezione del sito SSI era al minimo nei casi in cui si preferiva la chirurgia minimamente invasiva (OR=0.062)(0.006-0.628)(P=0.019). Inoltre, il tasso di sopravvivenza mediano complessivo per la coorte totale è stato di 38±3,1 (31-44) mesi. L'analisi multivariata ha mostrato che CEA (> 5 ng/mL) (HR 2,94) (1,337-6,492)) (P=0,007); stadio del tumore (P=0,034), stadio T(T4) (HR 1,91) (1,605-252,6) (P=0,02); metastasectomia/ablazione (HR 0,19) (0,077-0,520) (P=0,001); il numero di linfonodi metastatici rimossi (HR 1,08) (1,010-1,155) (P=0,025); impianto o nodulo tumorale (HR 2,71) (1,102-6,706) (P=0,03); resezione curativa (HR 2,40)(0,878-6,580)(P=0,042) come fattori che influenzano il tasso di sopravvivenza globale.

Conclusione: Il trattamento con Bevacizumab durante il periodo neoadiuvante nei casi mut-K(N)RAS, la tecnica chirurgica e le complicanze di grado 3 o superiore sono fattori di rischio per SSI sulla morbilità in pazienti con mut/wild-K(N)RAS sottoposti a cancro del colon-retto chirurgia. Inoltre, CEA (>5ng/mL), stadio del tumore, stadio T, metastasectomia/ablazione, numero di linfonodi metastatici rimossi, impianto/nodulo del tumore e resezione curativa sono fattori di rischio sul tasso di sopravvivenza globale.

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