

MEDICAL UNIVERSITY - PLEVEN
DEPARTMENT OF SURGICAL NURSING CARE

TSANKO IVELINOV YOTSOV, MD

ABSTRACT

Of a dissertation thesis for acquiring the educational and academic degree
DOCTOR of PHILOSOPHY

**RESEARCH ON THE ROLE OF INTRAOPERATIVE FLUORESCENT
ANGIOGRAPHY IN MINIMALLY INVASIVE COLORECTAL
SURGERY**

Academic supervisors:

Prof. Dobromir Dimitrov, MD, PhD

Assoc.prof. Svilen Maslyankov, MD, PhD

PLEVEN, 2023

The PhD student works as an assistant in the Healthcare Department at “Angel Kanchev” University of Ruse.

The dissertation thesis consists of 114 pages, 17 tables, 26 figures and 3 appendices. The bibliography includes 115 English titles.

The dissertation was approved and referred for defense by the large body of the Department Council of the Surgical Nursing Care Department at the Faculty of Healthcare, MU-Pleven.

The public defense will be held on 28.04.2023, at 13:00, in the “Ambroise Parè” Hall in MU-Pleven, in accordance with the regulations and conditions for acquiring academic degrees and taking academic positions in MU-Pleven, and based on Order №134/12.01.2023 by the Rector of MU-Pleven, in front of the following academic jury:

Chairman:

Assoc. prof Pencho Tonchev

Members:

Prof. Nikola Kolev

Prof. Rosen Dimov

Prof. Sergey Iliev

Assoc. prof Manol Sokolov

The materials for the dissertation thesis defense are available on the MU-Pleven site.

CONTENTS:

I.Introduction.....

II. Aim and objectives.....

III. Materials and methods.....

IV. Results.....

V. Conclusions.....

VI. Contributions.....

VII. Appendices.....

VIII. Scientific announcements and publications related to the dissertation thesis.....

ABBREVIATIONS USED:

ICG – Indocyanine Green

CRM+ - Positive Circumferential Resection Margin

AL – Anastomotic Leak

CRC – Colorectal Cancer

AJCC - American Joint Committee on Cancer

TME – Total Mesorectal Excision

PME – Partial Mesorectal Excision

TaTME – Transanal Total Mesorectal Excision

TNM-system – Tumor, Node, Metastasis system

ESMO – European Society of Medical Oncology

NCCN – National Comprehensive Cancer Network

ERAS – enhanced recovery after surgery

NIR – near infrared

FDA – Food and Drug Administration

IMA – Inferior Mesenteric Artery

ACL – Anocutaneous Line

OR – Odds Ratio

CI – Confidence Interval

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

CT – Computed Tomography

MRI – Magnetic Resonance Imaging

I. INTRODUCTION

Colorectal cancer, due to its high frequency, can be viewed as one of the socially significant diseases. Surgery is part and parcel of the total approach for treating this disease. Like any other surgery, interventions in the colorectal area are accompanied by complications – some of which resulting in serious consequences for the patients. Anastomotic leak (AL) after colorectal surgery is a complication that, in spite of long-standing research on it, has permanently been keeping high levels, leading to considerable morbidity and mortality, and worsening of the quality of life in patients. Neither should the financial burden of the treatment of an AL patient be underestimated. Intraoperative fluorescent perfusion angiography in colorectal surgery is a new but already widely popular method that aims at decreasing the percentage of this complication. Having in mind this, we researched the role, complications and safety of applying ICG perfusion fluorescent angiography in minimally invasive colorectal surgery.

II. PURPOSE AND OBJECTIVES

AIM

The dissertation thesis aims to study the role, safety and complications of applying the ICG perfusion fluorescent angiography in minimally invasive colorectal surgery.

OBJECTIVES:

1. Creating a protocol for application of ICG perfusion fluorescent angiography for the purposes of minimally invasive colorectal surgery;
2. Studying the effectiveness of the protocol for the application and introduction of the ICG perfusion fluorescent angiography in minimally invasive colorectal surgery;
3. Selecting a patient cohort for intraoperative application of ICG perfusion fluorescent angiography in minimally invasive colorectal surgery and assess the characteristics of the patients in it;
4. Studying the intraoperative, pathoanatomical and postoperative results in the cohort to assess AL when ICG perfusion fluorescent angiography is applied intraoperatively during minimally invasive colorectal surgery.

III. MATERIALS AND METHODS

1. Materials

The present dissertation thesis is based on a prospective multi-center study of patients undergone minimally invasive surgery for colorectal cancer in which fluorescent perfusion angiography was applied. The study was carried on in the Clinic of Surgical Oncology of UMHAT “Georgi Stranski”, MU-Pleven and the Clinic of General Surgery – II ward of UMHAT “Alexandrovska”, MU-Sofia. The PhD thesis is supported by the European Regional Development Fund

through the Operational Programme "Science and Education for Smart Growth" under contract №BG05M2OP001-1.002-0010-C01(2018-2023). The consumables used for its practical realization were provided through a scientific research project of MU-Pleven for 2020, registered under №13. Approvals for carrying out a scientific research experiment were obtained from the Ethics Committee of the Medical University – Pleven, № 647- University ethics committee/ 11.06.2020, the department head and the hospital manager. In cooperation with Clinic of General Surgery – II ward of UMHAT “Alexandrovskа”, scientific research project №7414/19.11.2021 was prepared on a topic similar to the one of the present dissertation thesis. The patients included in the research project were added to the cohort from the dissertation thesis. Пациентите включени в проучването по проекта бяха включени в кохортата (Appendix 1 – project documents dated, signed and stamped).

Data collection sheets for colon and rectal cancer (Appendices 2 and 3) were prepared and used for registering and systematizing the research data.

The ICG vials we used contained 25 mg powder for solution Verdye (Renew Pharmaceuticals Ltd, Germany).

Fig. 1 – ICG vial



Inclusion criteria for patients:

- Patients older than 18
- Planned surgical interventions
- Histologically proved colorectal cancer
- Minimally invasive procedure, even converted ones
- Signed informed agreement
- Preoperative CT scan or MRI
- Decision of the oncology committee for surgical treatment

Exclusion criteria for patients:

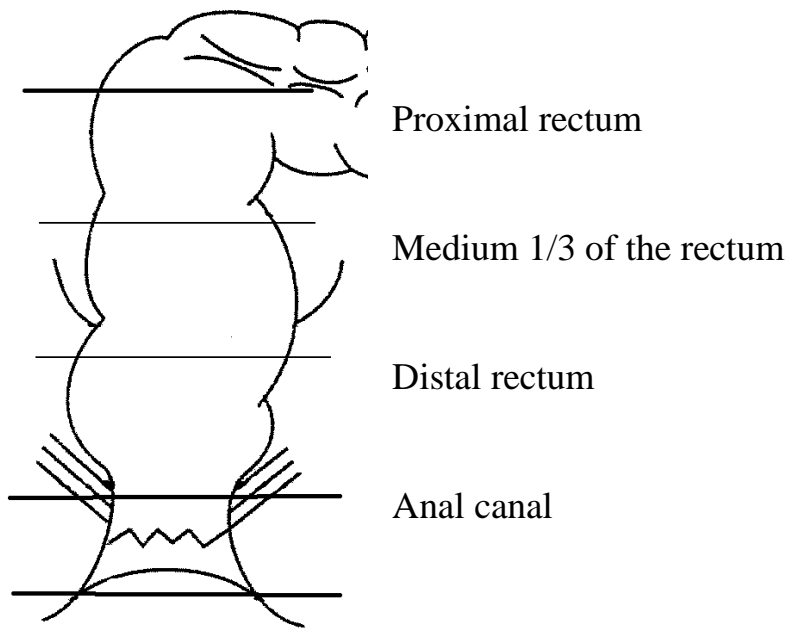
- Emergency surgical interventions
- Counterindications for general anesthesia
- Allergy to water-soluble iodinated contrast media
- Refusal of the patient to take part in the study

2. Methods

All the patients included in the study were subject to preoperative diagnostics and preparation. Diagnostics included clinical examination consisting of digital rectal exam, total colonoscopy with tumor biopsy, histological verification, laboratory tests, contrast enhanced CT or MRI and case discussion by an oncological committee. After a decision for surgical treatment was made, the patient was admitted in the clinic where he/she underwent preparation for surgery according to the ERAS protocol followed in the clinic. A day before the intervention the patient was given high-carbohydrate and high-protein medical foods (Nutricomp, Fresubin), Metronidazole (Flagyl) tabl. 3x250mg. p.o. Mechanical bowel preparation was done with 6 sachets of Endofalk, dissolved in 1.5 l of water. Perioperative prophylaxis with Cefuroxime x1500mg i.v. and Metronidazole x 1500mg was performed 30-60 minutes before skin incision and was discontinued after surgery. In case the operation lasted more than 4 hours, the Cefuroxime dose was administered again. A nasogastric tube was not inserted preoperatively or, if such was inserted, it was removed at the end of the intervention. An abdominal drain was placed only in cases of rectal resection and was removed at the 72nd hour at the latest. A transanal drain was fixed to the perineal skin in all post-rectal surgery patients.

The tumor location is important when dealing with rectal surgery. In classical surgical literature rectal cancer is described as a tumor starting at 15, 16 or 18 cm from the anocutaneous line. According to modern English sources a rectum is described as a structure consisting of three parts: low rectum – up to 6cm from the anocutaneous line; middle rectum – 7 to 11 cm from the anocutaneous line; and upper rectum – 12 to 15 cm from the anocutaneous line. We used the latter classification in defining the rectum and the rectal cancer location.

Fig. 2 – Parts of the rectum diagram.



Except for coloanal anastomoses in TaTME, most anastomoses were made with staplers. The staplers we used were GIA 80mm 3.5mm, EndoGIA 60mm 3.5mm, TA 45mm 4.5mm и EEA 32mm.

Fig. 3 – Pictures of the staplers used – GIA, EndoGIA, TA, EEA





In all cases of right localization of the colorectal cancer (CRC) we made latero-lateral anisoperistaltic ileotransverse anastomoses with GIA stapler 80mm 3.5mm placed through small enterotomies. The enterotomies for placing the the stapler were resected with another GIA stapler 80mm 3.5mm, together with the specimen. In cases of left-sided CRC we made termino-terminal colorectoanastomoses using the Knight-Griffen double stapling technique. To transect the distal resection line we used TA 45mm 4.5mm for extracorporeal anastomosis and EndoGIA 60mm 3.5mm for intracorporeal anastomosis. The circular stapler we used was always the same type – EEA 32mm.

The statistical data analysis was performed with the IBM SPSS 26.0 program. The following statistical methods were applied:

- (1) **Descriptive statistics** – includes all methods used for classifying data and obtaining their summarized characteristics.
 - a. **Quality data** – the descriptive statistics of quality variables includes: number and percentage of patients falling into the various categories
 - b. **Quantity data** – the description of frequency distribution consists in defining its form, mean values (arithmetic mean and median) and dispersion (standard deviation)
- (2) **Comparative deductive statistics** – used to check the hypotheses for difference

- a. **t-test** – parametric test for comparing the arithmetic means in two groups, used for interval and proportional data with normal distribution
 - b. **Mann-Whitney U** – a non-parametric test for comparing the medians of two groups, used for data without normal distribution
- (3) Associative deductive statistics** – used to check the hypotheses for association
- a. **χ^2 test of association (Chi-square of association)** – used for establishing the relation between two quality variables
 - b. **Phi (ϕ)** – for assessment of the strength of relation between two quality variables; applicable for cross tabulation with a table 2x2
 - c. **Cramer`s V** – for assessment of the strength of relation between two quality variables; applicable for cross tabulation with a table larger than 2x2
 - d. **Point-biserial correlation (r_{pb})** – for establishing the relation between two variables, when one variable is an interval or proportional variable and the other one is nominal (dichotomous)

(4) Table and graphic presentation of data

The consumable applied in perfusion angiography – indocyanine green – is available on the Bulgarian market at an affordable price. Unfortunately, it is not on the list of approved drugs and its use is regulated by the Bulgarian Drug Agency (BDA). To use it, we needed to prepare a protocol in accordance with Appendix 2 of Regulation 10 and submit it to BDA. The same protocol is required for other medicines used in the country, which belong to the group of “pharmaceuticals that are not allowed for use according to the list under Article 266a, §2, of the Drug use in Human Medicine Act“.

Indocyanine green is powder for solution in glass vials. The only condition for storage is that it is not exposed in direct sunlight. The substance is as easy to use as any other powder medicaments for intravenous application used in hospitals.

A number of experts from various fields in medicine, as well as the teams of clinical units at MU-Pleven and UMHAT “G.Stranski”, made direct or indirect contributions to the process of developing and implementing this academic work.

I owe special gratitude to my academic supervisors – Prof. Dobromir Dimitrov, MD, PhD, and Assoc.prof. Svilen Maslyankov, MD, PhD – for their unfailing support and instruction in working on the dissertation thesis. This work would not have been possible without the support of the teams of the Surgical Oncology Clinic, IIIrd Ward in Anesthesiology and Intensive Care, the Surgical Oncology Surgical Block, the Clinic of Pathological Anatomy, the clinical laboratory, the outpatient ward for oncological diseases, the Medical Oncology Ward and the Radiology Ward.

3. Equipment

The minimally invasive procedures performed were robot-assisted or laparoscopic.

We used a DaVinci Si robotic system, upgraded with a Firefly system for fluorescence imaging.

Fig. 4 – Robotic system.



The laparoscopic equipment we used consists of the following modules:

- Endoscopic processor (Camera unit) OLYMPUS OTV-S200;
- Light Source OLYMPUS CLV-S200-IR;
- FULL HD camera head OLYMPUS CH-S200-XZ-E;
- Optical telescope (laparoscope) OLYMPUS Trueview II;
- Insuflator OLYMPUS HighFlow UHI-4;
- HDTV OLYMPUS medical monitors;
- Recording system.

Fig. 5 – Laparoscopic equipment.



During the laparoscopic interventions an *Ethicon – Harmonic Ace* ultrasound scalpel was used.

Fig. 6 – An Ethicon – Harmonic Ace ultrasound scalpel.

HARMONIC®
Adaptive Tissue Technology
Intelligent energy delivery



IV. RESULTS

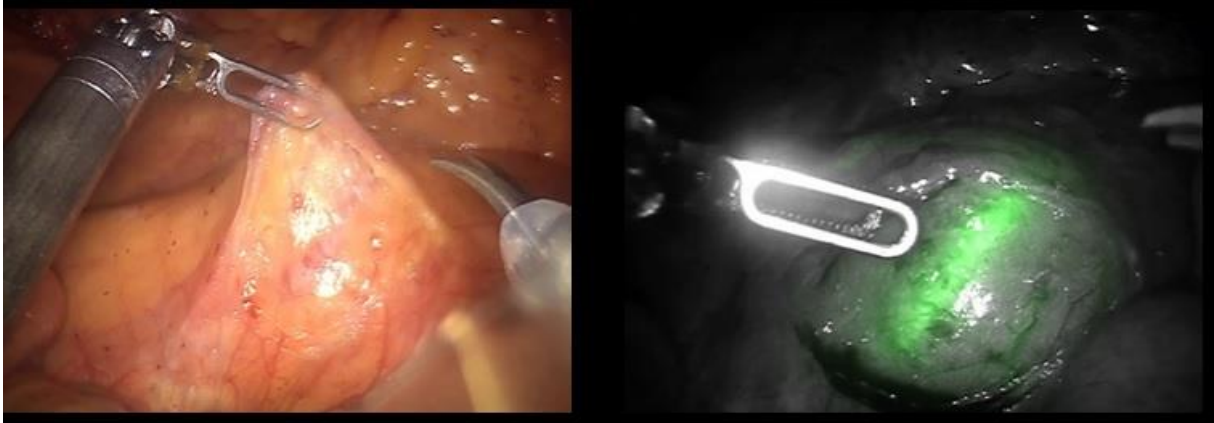
In the process of the academic thesis development, a cohort of 53 patients was selected; all of them underwent minimally invasive colorectal surgery with

ICG perfusion angiography. According to a data collection sheet made in advance, information on 45 characteristics was collected and divided into 3 groups: patient characteristics (13), intraoperative characteristics (15), pathologoanatomic characteristics (16) and one result related to anastomotic leak. Perfusion angiography is mainly applied to make an objective assessment of the anastomotic region, thus reducing the frequency of AL. For this reason comparison was made between the frequency of AL in the angiography cohort and a cohort from the previous 2 years, before the method was introduced in the clinic.

Results for the 1st objective – Creating a protocol for application of ICG perfusion fluorescent angiography for the purposes of minimally invasive colorectal surgery

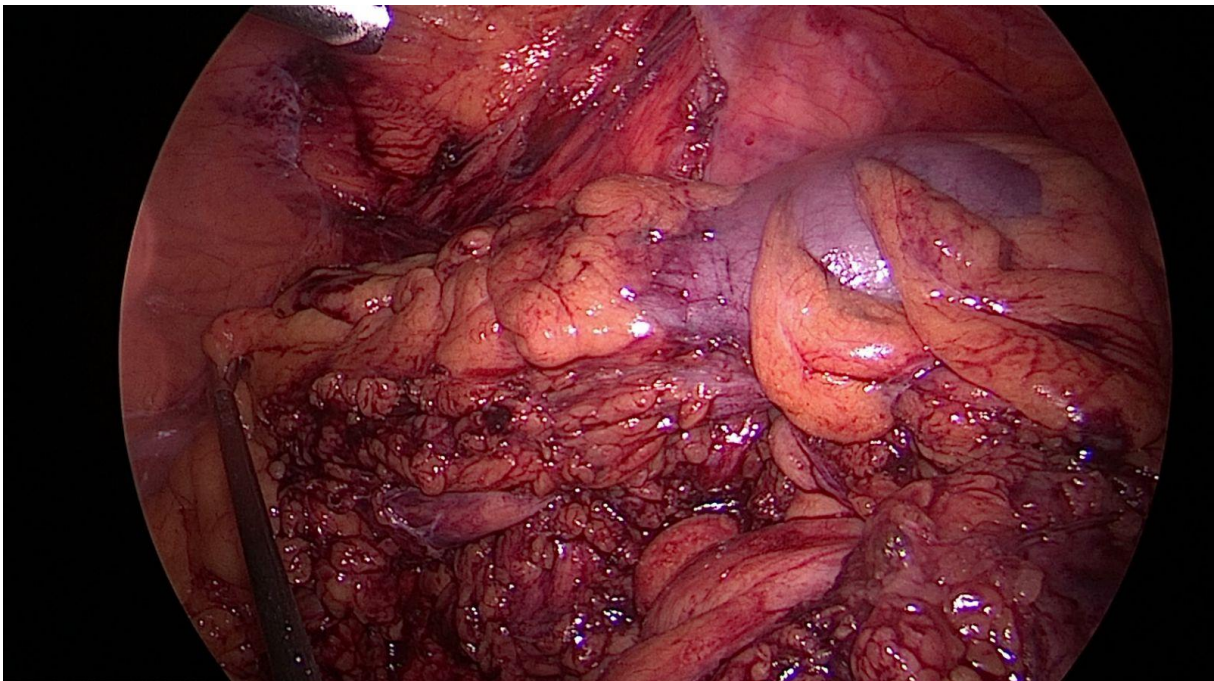
As there is not a universally accepted algorithm for performing fluorescent angiography, we created our own algorithm to use in the scientific experiment. According to it, 1 vial of 25mg of ICG is dissolved in 10 ml of sterile water for injections. The resulting solution is of 2.5mg/ml concentration. The first dose of the dye is 4ml and is administered intravenously at the beginning of the operation. Right after that 20ml of physiological serum is injected, again intravenously. The aim of giving the first dose is to visualize the position of the inferior mesenteric artery (IMA) and measure the time necessary for the dye to reach the colon (the appearance of fluorescence) i.e. the perfusion time. We labelled this time tICG1.

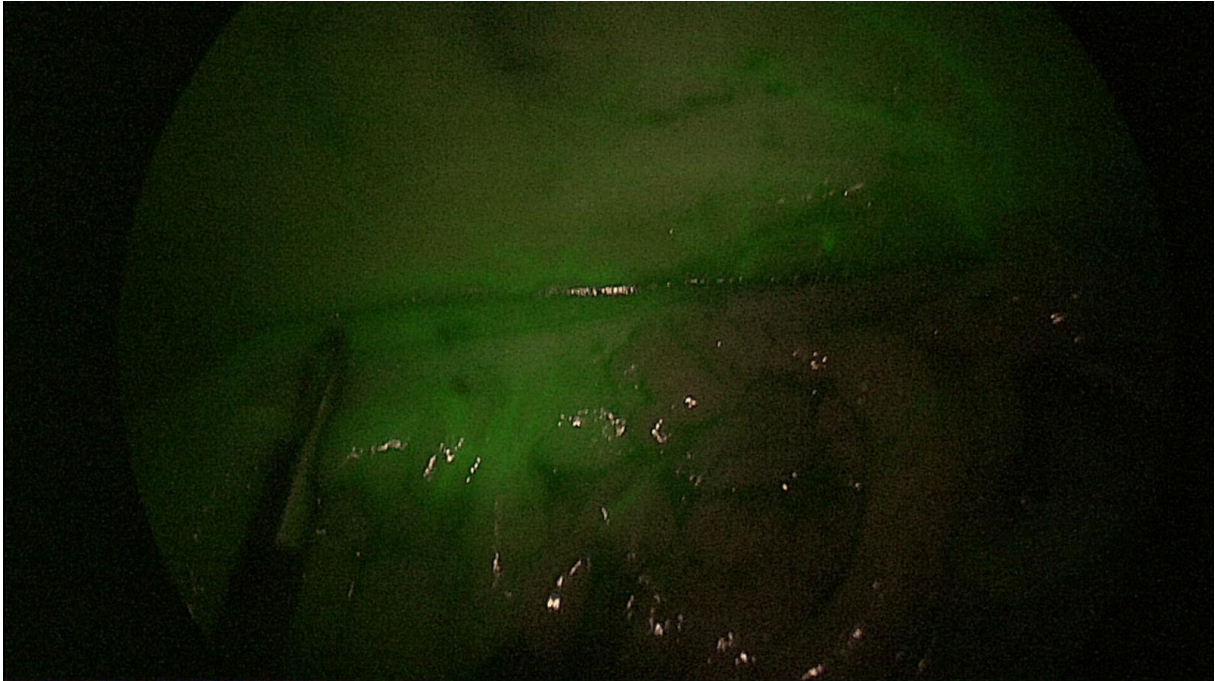
Fig. 7 – Visualization of IMA with ICG at the beginning of the intervention.



The second dose of the dye is another 4ml. It is injected after the IMA has been transected and the prospective resection line has been marked under white light. Right after the second ICG dose another 20 ml of physiological serum is injected. The aim of the second dose is to assess the blood supply of the colon after the IMA was transected and check if the area that had been marked for transection is supplied with blood. Again, the time until fluorescence appears is measured. We labelled that time tICG2.

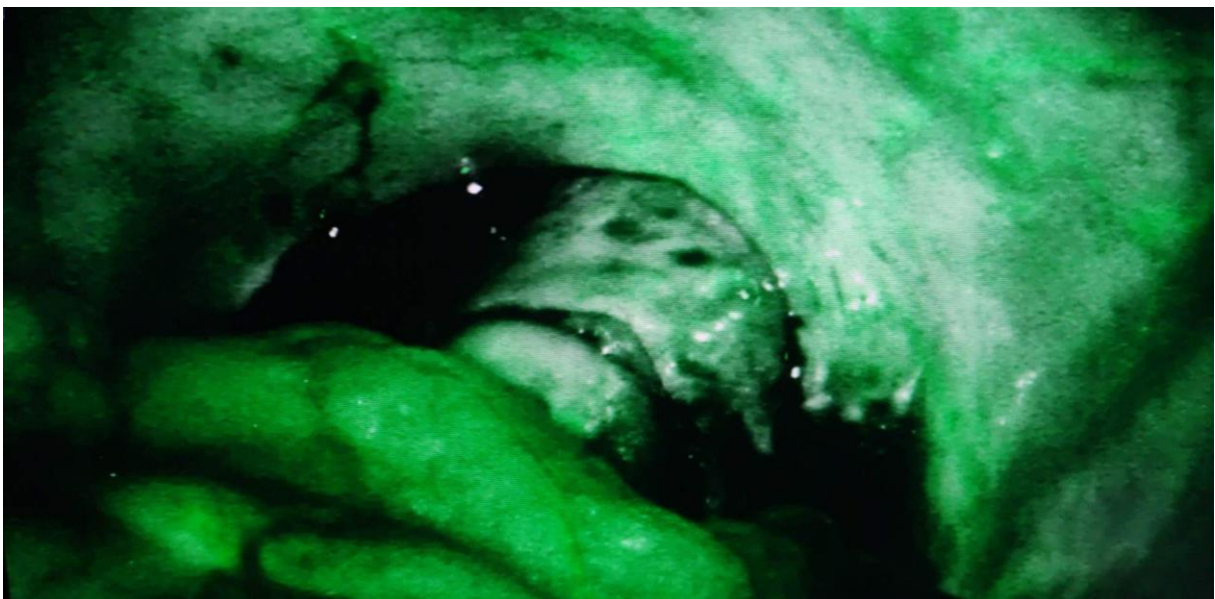
Fig. 8 – A picture from a laparoscopic operation to compare the image under white light to near-infrared (NIR) light after vessel transection. (tICG2)





The third dose of dye is 2ml and is administered after the anastomosis has been made. It is followed immediately by 20ml of physiological serum. The aim of the third dose is to visualize the blood supply in the whole anastomosis. This time was named tICG3.

Fig. 9 – Assessing the perfusion in the performed anastomosis tICG3



The measured times last from injecting the physiological serum to the

appearance of clear fluorescence in the tissues. The assessment is done through the filter of the NIR light camera.

Results for the 2nd objective - the effectiveness of the protocol for the application

To assess the effectiveness of the created protocol we used the simplicity of the method, the percentage of successful applications and the possibility for immediate interpretation of the result.

Most brands of laparoscopic surgery systems are equipped with filter modules for NIR light or a built-in NIR function with a LED light source. We used the OLYMPUS system in our OR which is equipped with such a module.

The patient cohort selected in the process of our experiment consisted of 53 participants. Perfusion angiography at the beginning of surgical intervention (tICG1) was performed in 50 patients. Assessment of the blood supply of the anastomosis segment (tICG2) was made for 51 patients and assessment of the anastomotic perfusion (tICG3) was made for 12 patients. This means that tICG1 was measured in 94,3% of the cases, tICG2 – in 96,22% of the cases, and tICG3 - in 22,64% of the cases. The patients without tICG1 measurement, were given tICG2 and tICG3 measurements. Therefore, perfusion assessment was made for all cohort patients and the method proved 100% applicable. For the 3 conversion cases, after the anastomosis segments had been prepared, assessment through fluorescence perfusion angiography was also made.

For the 9 cases where the site of anastomosis was changed, the decision was made intraoperatively, straight after performing the angiography and finding the fluorescence absent or insufficient in the prospective transection lines.

Results for the 3rd objective – Characteristics of the patients in the cohort:

The descriptive statistical analysis of the characteristics of patients showed their average age as 67.25 ± 10.866 years, with a median of 69 years, ranging from 35 to 84. Thirty-six (67,9%) of the patients were men. There was only one case (1.9%) of clinical obstruction. Neoadjuvant therapy had been performed in 17 patients (32,1%) with rectal locations of the cancer only; a fully pathological response had been observed in only 2 of them (3,8% of the whole cohort). Two patients (3,8% of the whole cohort) did not respond and 13 patients had imaging or clinical response. Seven of the patients (13,2%) had anamnesis for previous surgery for another malignant neoplasm before being diagnosed with colorectal cancer. The tumor in all 7 patients was left-sided, with 5 of the cases with rectal cancer. All patients had undergone preoperative CT or PET-CT. The patients in which the tumor was located in the middle or distal third of the rectum had undergone preoperative MRI of pelvis with contrast matter and gel administered transanally to fill the cavity of the rectum. Four of the patients (7,6%) gave information about a close relative with colorectal cancer. The mean values of preoperative albumin recorded for 50 out of 53 patients were 42.05 ± 5.146 g/L. Only 4 of the patients (7,5%) had albumin levels lower than 35 g/L, which is associated with greater risk of AL. The lowest recorded value was 32,6 g/L. Table 2 shows the relative percentage of patients with albumin lower than 35.

Table 2 - relative percentage of patients with albumin lower than 35

Албумин	Брой	%	Valid %
< 35	4	7,5	8,0
> 35	46	86,8	92,0
Общо	50	94,3	100,0
Липсващи	3	5,7	
Общо	53	100,0	

The assessment of anesthesia risk was done according to the classification of the American Society of Anesthesiologists (ASA). The greatest number of patients were ASA 3, 14 patients were ASA 2, and 11 patients were ASA 4. Forty-three patients (81,1%) had hypertension and 4 were with diabetes. Almost 30% of the patients in the cohort were smokers. Table 3 represents the summarized data from the descriptive analysis.

Table 3 – Results from the descriptive statistical analysis of the patient characteristics.

Характеристики	Пациенти (n=53)
Възраст (Mean ± SD) (Me; Min - Max)	67.25 ± 10.866 (69; 35 - 84)
Пол (брой, %)	53 (100.0%)
Мъже	36 (67.9%)
Жени	17 (32.1%)
Клинична обструкция (брой, %)	1 (1.9%)
Неoadювантно лечение (брой, %)	17 (32.1%)
Образен и клиничен отговор (брой, %)	17 (32.1%)
без отговор	2 (3.8%)
пълнен отговор	2 (3.8%)
регресия	13 (24.5%)
Други неоплазми (NM) (брой, %)	7 (13.2%)
СТ/РЕТ-СТ (брой, %)	53 (100.0%)
Фамилна обремененост (брой, %)	4 (7.6%)
Албумин (Mean ± SD) (Me; Min - Max)	42.05 ± 5.146 (42.05; 32.60 - 58.70)
АСА (Me; Min - Max)	(3; 2 – 4)
АХ (брой, %)	43 (81.1%)
Диабет (брой, %)	10 (18.9%)

Тютюнопушене (брой, %)	15 (28.3%)
------------------------	------------

Results for the 4rth objective – studying the intraoperative, pathoanatomical and postoperative results in the cohort

From the 53 patients who underwent surgery, 39 (the predominant number) had rectal resections with either total mesorectal excision (20 patients) or partial mesorectal excision (21 patients). The difference between the total number of rectal resections and the sum of TME and PME is due to 2 cases with distal sigmoid colon cancer with partial resection of the upper part of the rectum. Sigmoidectomy was performed in 7 patients, right hemicolectomy in 4 patients, 1 subtotal colectomy, 1 left hemicolectomy and 1 abdominoperineal resection. In all cases of colon cancer surgery was performed as total mesocolic escision. The kinds of minimally invasive techniques used in the cohort was balanced, with 25 robot-assisted resections (47.2%) and 28 laparoscopic ones (52.8%). All robot-assisted resections were performed in rectal cancer cases. Three of the cases (5.7%) necessitated conversion – one in a robot-assisted intervention for rectal cancer in the middle third of the rectum and the two conversions in the laparoscopic arm were for rectal cancers in the proximal and middle third respectively. High vessel ligation was performed in 35 пациента (66% of the whole cohort and 65% in left-sided locations). Splenic flexure mobilization was performed in 18 patients, 17 of which during surgery for rectal cancer and 1 – during left hemicolectomy. This means that in almost half of the interventions for rectal cancer (43.5%) splenic flexure had to be mobilized. The measurements after the perfusion angiography showed mean time ICG1 15.70 ± 7.338 seconds ranging from 7 to 35, and mean time ICG2 - 30.92 ± 10.288 seconds ranging from 12 to 65. In 9 of the patients (17%) the anastomosis region had to be changed.

In 6 of them the correction of the resection boundary was in the proximal part of the bowel, in 2 cases – in the distal part of the rectal stump, and in 1 patient – in both places. For 48 of all anastomoses we used staplers; the rest were made

by hand. The types of anastomoses made in the cohort were as follows: colorectal anastomosis in 42 patients (79.2%); ileotransversoanastomosis – in 4 patients (7.5%); coloanal anastomosis – in 3 patients (5.7%); colo-colic anastomosis – in 2 patients (3.8%); and ileorectal anastomosis – in 1 patient (1,9%). In accordance with the protocol of the clinic, in 37 out of 39 patients with rectal location of the tumor (94.8%) a transanal drain was placed. Defunctioning stoma was constructed in 17 patients, i.e. one-third of all that underwent surgery (32,1%). In all stoma cases the tumor was located in either the middle or the distal third of the rectum, with one exception where the tumor was located in the high rectum. The following table shows the results from the statistical analysis:

Table 6 – Results from the operative characteristics of patients:

Оперативен показател	Пациенти (n=53)
Вид операция (брой, %)	53 (100.0%)
РРА	39 (73.6%)
Сигмоидектомия	7 (13.2%)
Дясна хеми	4 (7.5%)
Субтотална	1 (1.9%)
Класическа лява	1 (1.9%)
АПР	1 (1.9%)
Вид МИХ (брой, %)	53 (100.0%)
Роботска	25 (47.2%)
Лапароскопска	28 (52.8%)
Цялостна мезоректална/ мезоколична ексцизия/ парциална мезоректална (брой, %)	53 (100.0%)
ТМЕ	20 (37.7%)
СМЕ	12 (22.6%)
РМЕ	21 (39.6%)
Конверсия (брой, %)	3 (5.7%)

Оперативен показател	Пациенти (n=53)
Високо лигиране (брой, %)	35 (66.0%)
Мобилизация на лиенална флексура (брой, %)	18 (34.0%)
Трансанален дренаж (брой, %)	39 (73.6%)
Stoma (брой, %)	17 (32.1%)
Анастомоза (брой, %)	52 (98.1%)
Съшивател	48 (90.6%)
На ръка	4 (7.5%)
Вид анастомоза (брой, %)	52 (98.1%)
Коло-ректо анастомоза	42 (79.2%)
Илеотрансверзо	4 (7.5%)
Коло-ано анастомоза	3 (5.7%)
Коло-коло анастомоза	2 (3.8%)
Илео-ректо анастомоза	1 (1.9%)
ICG1 (n=50) (Mean ± SD) (Me; Min - Max)	15.70 ± 7.338 (12.50; 7 – 35)
ICG2 (n=51) (Mean ± SD) (Me; Min - Max)	30.92 ± 10.288 (31; 12 – 65)
ICG3 (n=12) (Mean ± SD) (Me; Min - Max)	40.33 ± 17.079 (36; 28 – 90)
Промяна на мястото на анастомозата (брой, %)	9 (17.0%)
Проксимално	6 (11.3%)
Дистално	2 (3.8%)
И на двете страни	1 (1.9%)

The pathologoanatomical indicators from the data collection sheet were divided in 2 groups: macroscopic and microscopic assessment.

Analyzing the tumor location along the colon, the one that was most often found in the cohort was proximal rectum – 18 patients (34%). The rest of the

locations had the following frequency: mid third of the rectum – in 13 patients (24,5%), sigmoid colon – 9 patients (17%), distal rectum – 8 patients (15,1%), ascending colon – 4 patients (7,5%) and descending colon – 1 patient (1,9%). In more than half of the patients the macroscopic appearance of the formation was polypoid – 34 patients (64.2%). Infiltration through the wall was observed in 5 cases (9,4%). The mean distance to the distal resection line was 62.08 ± 56.240 mm, with a median of 50mm ranging from 20 to 280mm. The mean distance to the proximal resection line was 176.23 ± 128.680 mm, with a median of 150mm ranging from 70 to 1000 mm. The quality of the mesorectal and mesocolic excisions was found complete in all cases. The pathologists reported detected lymph nodes in the mesocolon and mesentery in 41 patients. The mean number of detected lymph nodes was 8.37 ± 7.599 , with a median of 7 lymph nodes ranging from 0 to 40. We did not have a case of infiltration in the proximal or distal resection line. As to the grade of malignancy, the greatest number was that of tumors defined as moderately differentiated G2 – 46 of the cases (86,8%). Invasion of a blood vessel was observed in one patient. The same applies for infiltration of lymph vessels. Perineural invasion was found in 3 patients. Table 9 summarizes the results from the descriptive analysis and pathologic-anatomical characteristics.

Table 9 – Results from the pathologic-anatomical characteristics.

Патологоанатомичен показател	Пациенти (n=53)
Локализация на продължението (брой, %)	53 (100.0%)
Ректум прок	18 (34.0%)
Ректум среден	13 (24.5%)
Сигма	9 (17.0%)
Ректум дистален	8 (15.1%)
Асценденс	4 (7.5%)
Десценденс	1 (1.9%)

Локализация напречен срез (брой, %)	53 (100.0%)
Циркулярно	20 (37.7%)
Предна	12 (22.6%)
Задна	12 (22.6%)
Мезоколична	5 (9.4%)
Антимезоколична	4 (7.5%)
Макроскопски вид (брой, %)	53 (100.0%)
Полипоиден	34 (64.2%)
Язвено-инфилтративен	12 (22.6%)
Язвен	2 (3.8%)
Дифузно инфилтриращ	2 (3.8%)
Некласифициран	2 (3.8%)
Повърхностен	1 (1.9%)
Инфилтрация през стената (брой, %)	5 (9.4%)
Разстояние до дистална линия (Mean \pm SD) (Me; Min - Max)	62.08 \pm 56.240 (50; 20 – 280)
Разстояние до проксимална линия (Mean \pm SD) (Me; Min - Max)	176.23 \pm 128.680 (150; 70 – 1000)
Качество на мезоколичната ексцизия (брой, %)	53 (100.0%)
Брой лимфни възли (n=41) (Mean \pm SD) (Me; Min - Max)	8.37 \pm 7.599 (7; 0 – 40)
Инфилтрация на дистална линия (брой, %)	0 (0.0%)
Инфилтрация на проксимална линия (брой, %)	0 (0.0%)
Степен на малигненост (брой, %)	52 (100.0%)
G1	5 (9.4%)
G2	46 (86.8%)
G3	1 (1.9%)
Инвазия в кръвоносни съдове (брой, %)	52 (98.1%)

Инвазия в лимфни съдове (брой, %)	1 (1.9%)
Перинеурална инвазия (брой, %)	3 (5.7%)
Инсуфициенция (брой, %)	0 (0.0%)

Staging of tumors was done according to the TNM classification of the American Joint Committee on Cancer (AJCC). The stage that was most often found in our cohort was T3N0M0. That was the stage in 19 patients (35.8%). The second in frequency was the T2N0M0 stage found in 11 patients (20.7%).

In our cohort there was not even one case of AL (0%). This rate was compared to the AL rate for the 2 years before introducing the method in the clinic – for 52 minimally invasive interventions performed there were 3 anastomotic leak cases, or 5.76%.

Besides the descriptive statistical analysis, we did also associative deductive analysis. We compared data about splenic flexure mobilization in left-sided locations and the time tICG2 until fluorescence appeared in the transection line. Chi-square test was used for that purpose. No statistically significant relation was found between splenic flexure mobilization and tICG2 ($\chi^2=26.941$, $df=22$, $p=0.213$).

Table 10 – Distribution of the perfusion time tICG2 in relation to splenic flexure mobilization

Мобилизация на лиенална флексура	ICG2																				Total			
	12	13	20	22	25	27	28	29	31	32	33	34	35	36	37	40	41	42	43	45		47	50	65
да	2	1	2	1	1	1	0	1	1	2	0	1	1	1	0	1	0	0	1	0	0	0	0	17
не	0	0	4	0	6	0	2	0	0	2	1	0	3	0	3	1	1	1	0	1	2	1	1	29
Total	2	1	6	1	7	1	2	1	1	4	1	1	4	1	3	2	1	1	1	1	2	1	1	46

Fig. 10 – The chart showing the distribution of tICG2 in groups of 10 seconds each

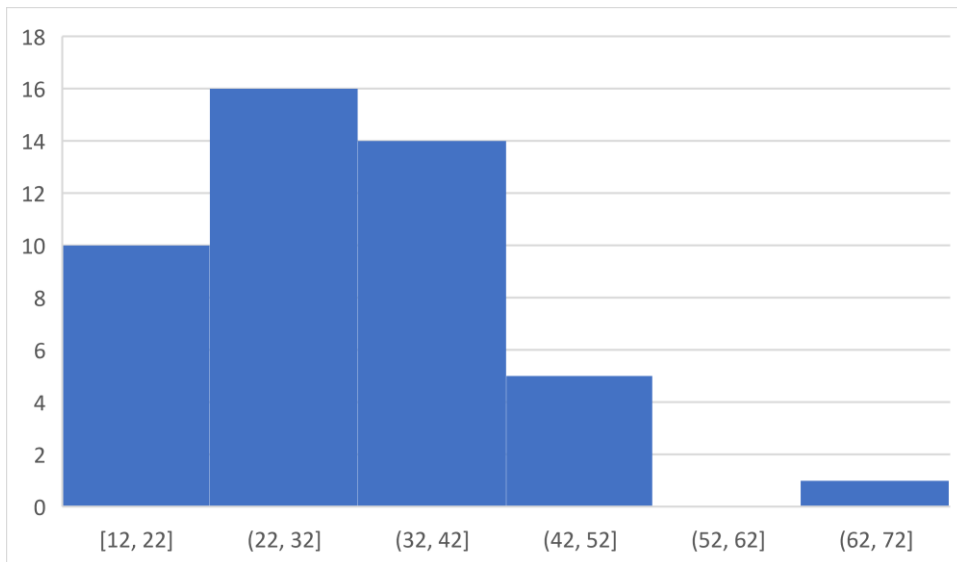
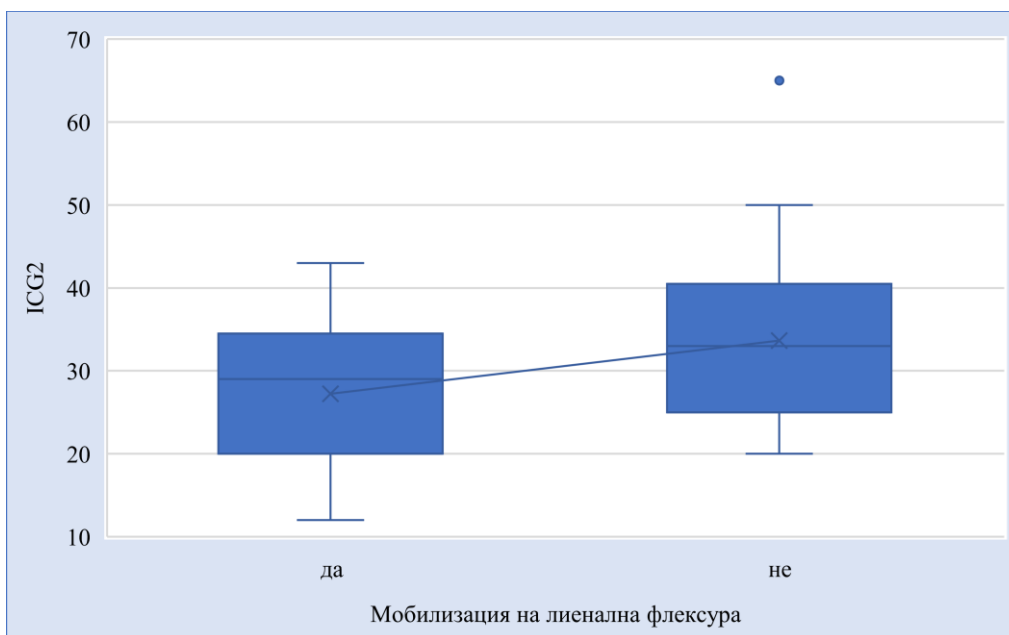


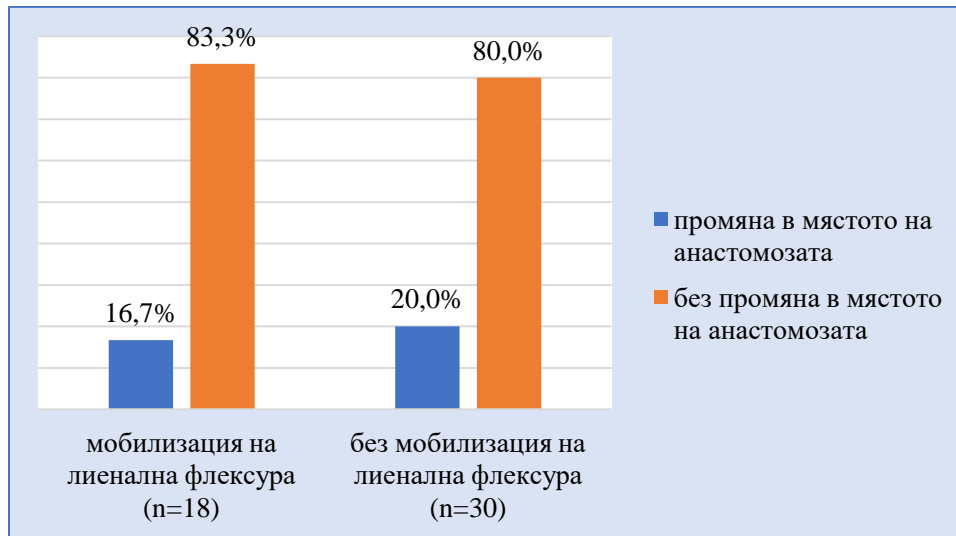
Fig. 11 – Box-plot diagram demonstrating the distribution of patients according to tICG2 and their median in patients with or without splenic flexure mobilization respectively.



We applied the same test to check for relation between flexure mobilization and change of the transection line. No statistically significant correlation was found between the variables ($\chi^2=0.082$, $df=1$, $p=0.775$, $\Phi=0.041$).

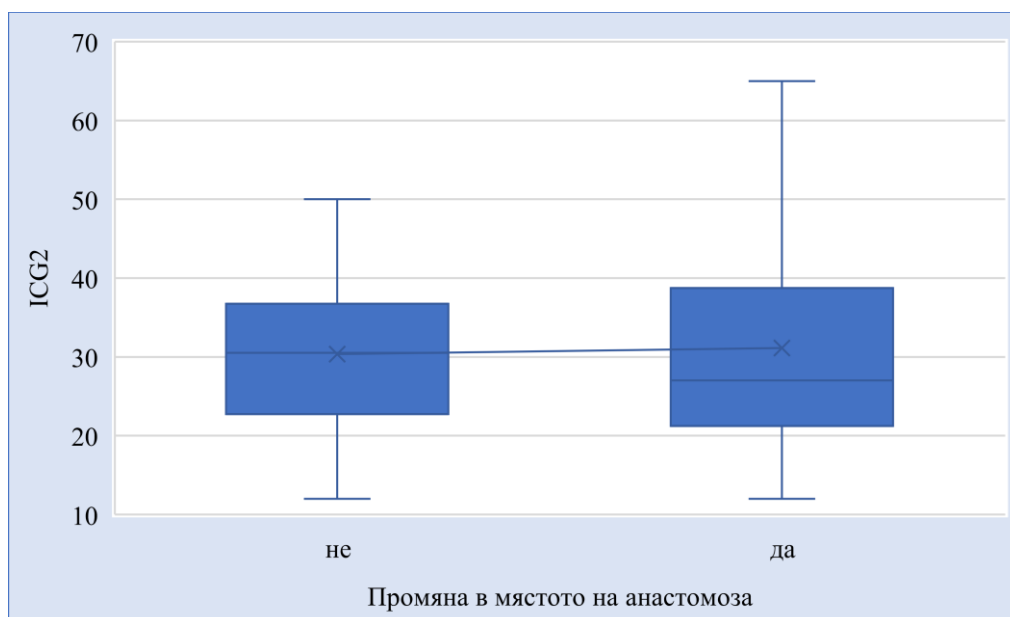
Fig.12 – Bar chart illustrating performed splenic flexure mobilization in relation

to the need to change the transection line.



Using point-biserial correlation we checked for relations between tICG2 and the change of transection area. No statistically significant correlation was found between the variables ($r_{pb}=-0.079$, $N=51$, $p=0.580$).

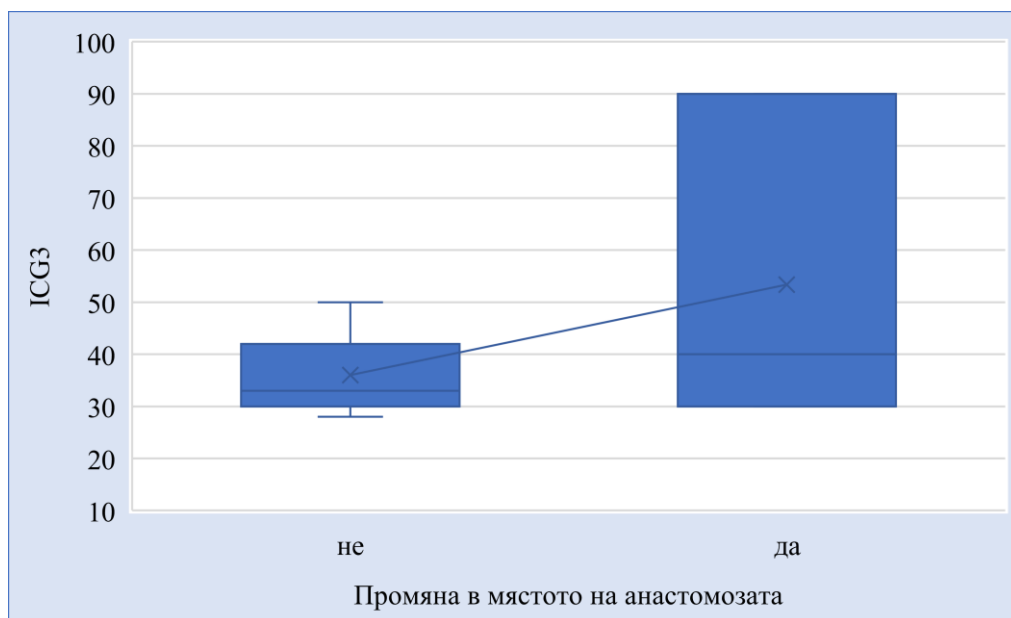
Fig. 13 - Box-plot diagram demonstrating the distribution of tICG2 and the change of the anastomosis site.



Student's t-test was applied as well and it did not show any statistically significant difference in ICG2 in cases with or without change of the anastomosis site ($t=0.390$, $df=9.132$, $p=0.705$).

The same analysis was done for tICG3 and the change of transection area. Once again, no statistically significant correlation was found between the variables ($r_{pb}=-0.459$, $N=53$, $p=0.133$).

Fig. 14 - Box-plot diagram demonstrating the distribution of tICG3 and change of the anastomosis site



The Mann-Whitney test did not find any statistically significant difference between ICG3 in patients with or without change of the anastomosis site. ($U=9.000$, $N=12$, $z=-0.849$, $p=0.482$)

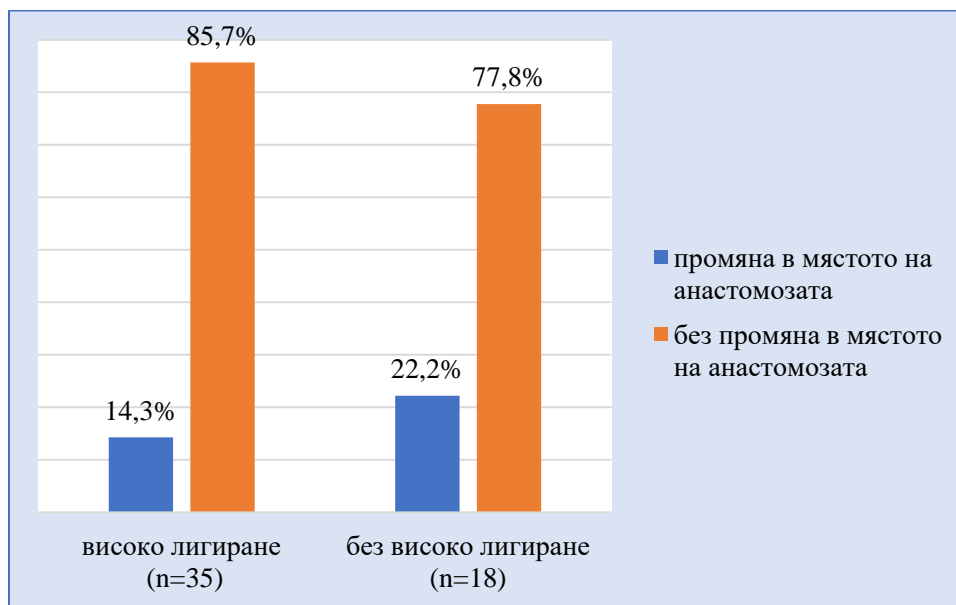
We carried out statistical analysis with Chi-square test to check for correlation between high ligation and change of the transection site. Phi test was used to assess the significance of relation. No statistically significant correlation was found between the variables ($\chi^2=0.531$, $df=1$, $p=0.357$, $\Phi=0.100$).

Table 11 - Crosstabulation of the data on high vessel ligation and change of the anastomosis site.

<p>Високо лигиране * Промяна в мястото на анастомозата Crosstabulation</p>

		Промяна в мястото на анастомозата		Total
		промяна в мястото на анастомозата	без промяна в мястото на анастомозата	
Високо лигиране	високо лигиране	5	30	35
	без високо лигиране	4	14	18
Total		9	44	53

Fig. 15 – bar chart illustrating data on high vessel ligation and change of anastomosis site

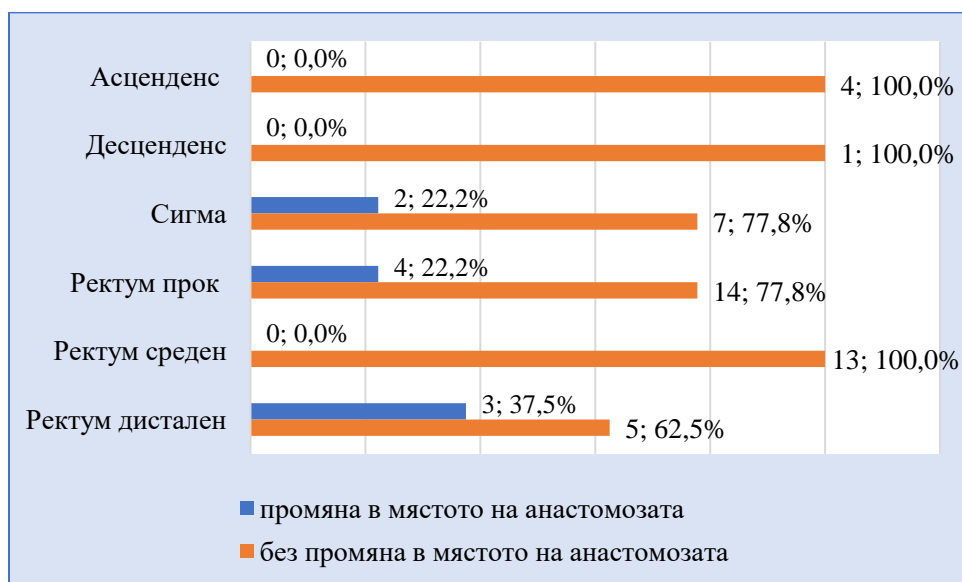


Another statistical analysis was done on the correlation between change of the transection site and location of tumor along the colon. No statistically significant difference was found between the variables ($\chi^2=6.597$, $df=5$, $p=0.252$, Cramer`s V=0.353).

Table 12 - Crosstabulation of the data on tumor location along the colon and the need to change the anastomosis site.

Локализация на продължението * Промяна в мястото на анастомозата Crosstabulation				
		Промяна в мястото на анастомозата		Total
		промяна в мястото на анастомозата	без промяна в мястото на анастомозата	
Локализация на продължението	Асценденс	0	4	4
	Десценденс	0	1	1
	Сигма	2	7	9
	Ректум прок	4	14	18
	Ректум среден	0	13	13
	Ректум дистален	3	5	8
Total		9	44	53

Fig. 16 – bar chart showing the data on tumor location in the colon and the need to change the anastomosis site



In the subgroup analysis, statistically significant difference was found between the number of cases with and without change of the anastomotic site, when the tumor was located in the proximal rectum. ($\chi^2=5,556$, $df=1$, $p=0.018$).

No undesired side effects from the drug use were observed in ICG perfusion angiography.

V. CONCLUSIONS

1. We proved the benefit of applying ICG fluorescent perfusion angiography during minimally invasive colorectal surgery, with 100% decrease in the AL frequency in comparison to the control group.
2. We proved the effectiveness of the created protocol, with the ICG fluorescent perfusion angiography applied to all patients in the cohort, including the three conversion cases.
3. The selected cohort of patients with performed ICG perfusion angiography was comparable to the control group. The analysis of the basic indicators of patients showed that 30% of them had a combination of 2 or more risk factors for AL.
4. The results from the analysis of the research data showed good clinical outcomes after ICG fluorescent perfusion angiography – no AL or adverse reactions to the medicament were observed.
5. Specimen evaluation showed high quality of the mesorectal (mesocolic) excision – no positive resection lines were detected.

VI. CONTRIBUTIONS

1. Contributions to scientific theory
 - The data available in literature were studied and an algorithm was created for the intraoperative application of ICG fluorescent perfusion angiography in minimally invasive colorectal surgery.
2. Contributions to scientific practice
 - A protocol for ICG perfusion angiography was introduced in the routine minimally invasive colorectal surgery;

- Studied were the clinical outcomes from the application of ICG perfusion angiography in minimally invasive colorectal surgery;
- Studied were the preoperative characteristics of patients and the postoperative characteristics of the specimens from the performed minimally invasive colorectal interventions.

VII. APPENDICES

- *Appendix 1*

Проспективно кохортно проучване върху 28 пациента с поне един рисков фактор за развитие на инсуфициенция на анастомозата при минимално инвазивна колоректална резекционна хирургия с първична анастомоза.

3. Имунофлуоресценция с ICG

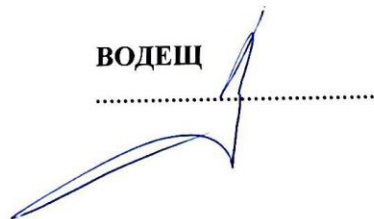
Интраоперативно ще се проведе микроангиография чрез имунофлуоресценция с ICG. За целта ще бъдат въведени в системното кръвообращение чрез абокат 25мг ICG. Тъй като ICG се свързва с плазмените белтъци остава само в кръвообращението и не излиза в междуклетъчното пространство. Със специална мултидетекторна камера за светлина с дължина на вълната близка до инфрачервената, в случая 820nm, ще се наблюдава дали участъкът определен за извършване на анастомоза е кръвоснабден.

Очаквани научни резултати и характеристика на научния принос (практическа или теоретична насоченост). Очакваните резултати са за намиране на доказателства за снижаване на честотата на инсуфициенциите на анастомозите при минимално инвазивна резекционна хирургия по повод колоректален карцином и внедряването на алгоритъм за използването на ICG в клиничната ни практиката.

5. РАБОТНА ПРОГРАМА:

- Литературния обзор по проблема
- Разработване на изследователски фиш
- Внасяне на проекта в Комисия по етика на научно-изследователската дейност.
- Въвеждане на метода в клиника по онкологична хирургия към УМБАЛ „Д-р Г. Странски“ Плевен
- Проследяване на пациентите за 30 дни
- Събиране на данните
- период на проследяване на пациентите
- Статистическа обработка и анализ на данните.

ВОДЕЩ



ИЗСЛЕДОВАТЕЛ:

(подпис)

Дата: 02.04.20

КОНТАКТНА ИНФОРМАЦИЯ

Посочете данните на лицето, към което Комисията по етика на научно-изследователската дейност при МУ-Плевен трябва да се обръща за допълнителна информация по научното изследване.

Име:	Мартин Караманлиев		
Длъжност:	Специализант и докторант по обща хирургия		
Адрес:	Ул. „Георги Кочев” 8А, УМБАЛ „Г. Странски“ – ЕАД, гр. Плевен		
Телефон:	0894242247	Е-mail:	martinkaramanliev@gmail.com

Подпис на главния изследовател:

Секретар на КЕНИД:

ПИСМЕНО СЪГЛАСИЕ НА РЪКОВОДИТЕЛЯ НА ЛЕЧЕБНОТО ЗАВЕДЕНИЕ

СЪГЛАСИЕ

**ПО ЧЛ. 87, АЛ. 3 ОТ ЗАКОНА ЗА ЛЕКАРСТВЕНИТЕ ПРОДУКТИ В ХУМАННАТА
МЕДИЦИНА**

Аз, долуподписаният проф. Т. Делийски, в качеството си на ръководител клиника по онкологична хирургия към УМБАЛ 'Д-р Георги Странски' ЕАД, Ул. Георги Кочев 8А, Плевен, България,

С настоящето давам съгласие за:

Провеждане на проучване „Проучване влиянието на интраоперативната микроангиография с индоцианин грийн върху интраоперативните и постоперативните резултати при пациенти с колоректален карцином, оперирани чрез минимално инвазивен подход“, организирано от екипа на клиниката по онкологична хирургия УМБАЛ „Г.Странски“ – Плевен на възрастни пациенти подложени на елективна резекционна хирургия при пациенти с карцином на ректума и извършване на интраоперативната микроангиография с индоцианин грийн

което ще се проведе в Клиника по онкологична хирургия към УМБАЛ 'Д-р Георги Странски' ЕАД, Ул. Георги Кочев 8А, 5800, Плевен, България, с главен изследовател доц. Добромир Димитров

Клиничното изпитване ще се проведе след одобрение на Комисия по етика на научно-изследователската дейност към МУ-Плевен.

Дата: 02.04.20


(подпис и печат)
/проф. Т. Делийски/



ПИСМЕНО СЪГЛАСИЕ НА РЪКОВОДИТЕЛЯ НА ЛЕЧЕБНОТО ЗАВЕДЕНИЕ

СЪГЛАСИЕ

ПО ЧЛ. 87, АЛ. 3 ОТ ЗАКОНА ЗА ЛЕКАРСТВЕНИТЕ ПРОДУКТИ В ХУМАННАТА МЕДИЦИНА

Аз, долуподписаният доц. Цветан Луканов, в качеството си на Изпълнителен Директор на УМБАЛ 'Д-р Георги Странски' ЕАД, Ул. Георги Кочев 8А, Плевен, България,
С настоящето давам съгласие за:

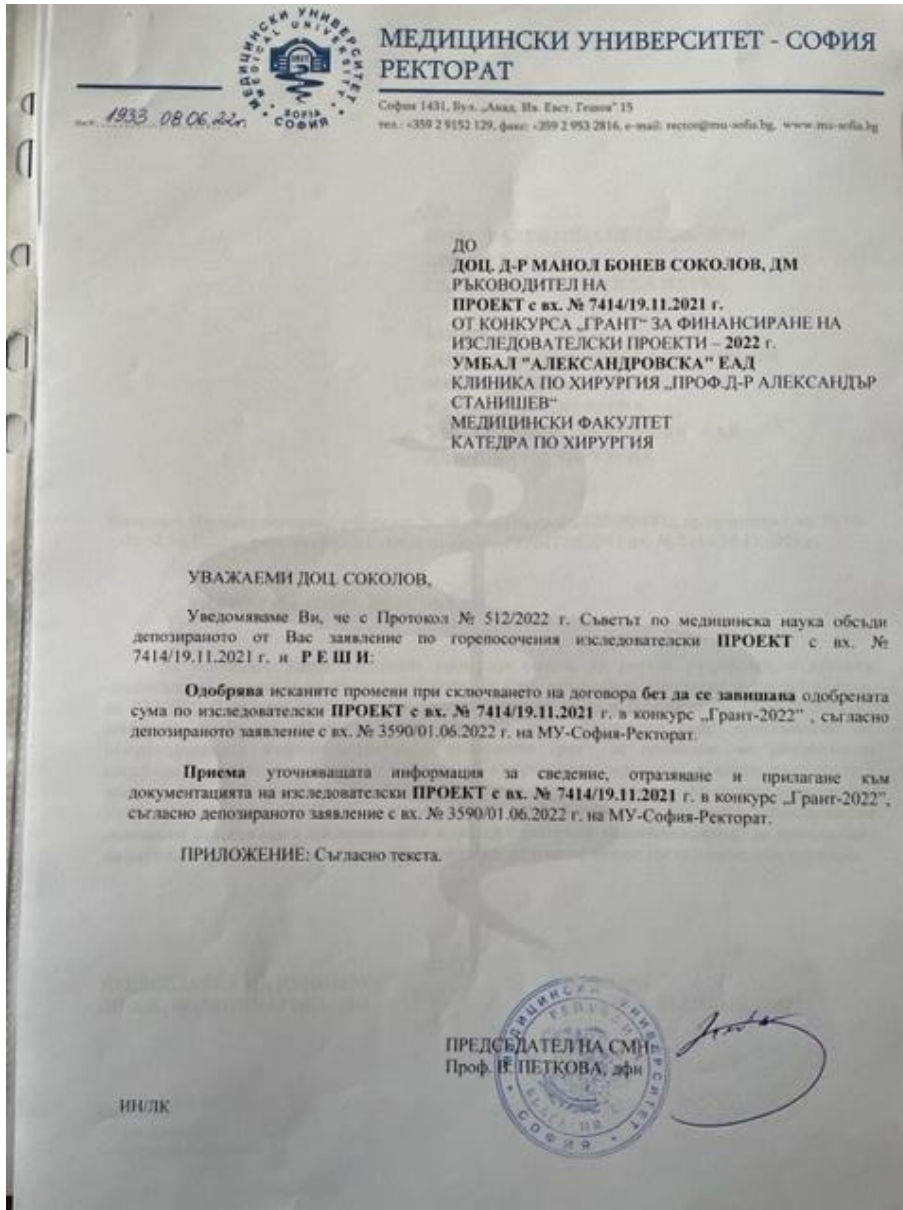
Провеждане на проучване „Проучване влиянието на интраоперативната микроангиография с индоцианин грийн върху интраоперативните и постоперативните резултати при пациенти с колоректален карцином, оперирани чрез минимално инвазивен подход“, организирано от екипа на клиниката по онкологична хирургия УМБАЛ „Г.Странски“ – Плевен на възрастни пациенти подложени на елективна резекционна хирургия при пациенти с карцином на ректума и извършване на интраоперативната микроангиография с индоцианин грийн

което ще се проведе в Клиника по онкологична хирургия към УМБАЛ 'Д-р Георги Странски' ЕАД, Ул. Георги Кочев 8А, 5800, Плевен, България, с главен изследовател доц. Добромир Димитров

Клиничното изпитване ще се проведе след одобрение на Комисия по етика на научно-изследователската дейност към МУ-Плевен.

Дата:





- Appendix 2

DATA COLLECTION SHEET FOR COLON CANCER SURGERY WITH ICG

- Name..... M/ F
- age. Case history..... BMI:.....
- Clinical diagnosis:
.....
- Clinical obstruction: NO/YES

- Neoadjuvant therapy performed: NO/YES
- 1. Clinical and imaging response. 2. No response. 3. Complete pathological response. 4. Regression (Downsizing).....
- Other concomitant/past neoplasms: NO/YES Specify.....
- Preoperative CT or PET-CT: NO/YES
- Genetic predisposition: NO/YES
- Serum albumin:..... ASA: Hypertension: : NO/YES Diabetes: : NO/YES Smoking : NO/YES
- Tumor markers:.....
- Type of surgery.....
- Total mesocolic excision: NO/YES
- High ligation of the vessel: NO/YES
- Splenic flexure mobilization: NO/YES
- Transanal drain: NO/YES
- Anastomosis: with a stapler/ by hand
- Type of anastomosis:.....
- **Immunofluorescence data:**
- Time until the first fluorescence appearance:
- Angiography at the beginning:.....
- Perfusion angiography:.....
- Anastomosis:.....
- Change of the anastomosis location: NO/YES Direction:.....
- **Specimen sent:**.....

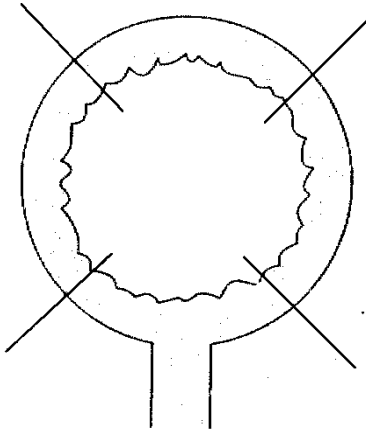
-
.....

-
-
-
-

- **Tumor location**

- A. Mesocolic wall B. Antimesocolic wall

1. Caecum 2. Colon ascendens 3. Flexura coli dex.



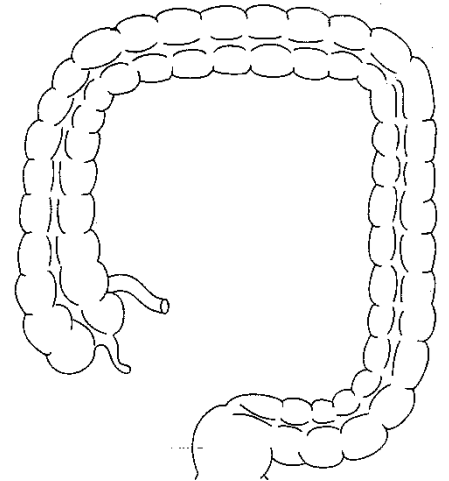
- C. Anterior wall D. Posterior wall

4. Colon transversum 5. Flexura coli sin.

- (for colon transversum – C. Inferior D. Superior)

6. Colon descendens 7. Colon sigmoideum

- E. Circumferential location



-
-
-
-

- Macroscopic assessment of the fresh specimen: Tumor:

dimensions...../.....cm (longitudinal/transversal)

Macroscopic appearance: 1.superficial 2.polypoid 3. ulcerous 4.

Infiltrating ulcer 5. Diffusely infiltrating 6.unclassified

.....

- Infiltration through the wall: NO/YES 1. Serosa 2. Pericolonic fascia (for locations in a wall without serosa) 3. Adjacent organs or

structures:.....

- Distance to: distal resection line.....cm; proximal resection line.....cm

- Assessment of the quality of the mesocolic excision: 1. Complete; 2. Near-complete; 3. Incomplete
- TNM: cT....N....M... pT...N...M...
- Number of lymph nodes:.....
- Infiltration of the resection line:
 - 1. Distal:.....
 - 2. Proximal:.....
- Malignancy grade: G1 G2 G3
- Invasion of blood vessels: NO/YES
- Invasion of lymph vessels: NO/YES
- Perineural invasion: NO/YES
- Anastomotic leak: NO/YES
-
-
- Date:.....
- Surgeon:

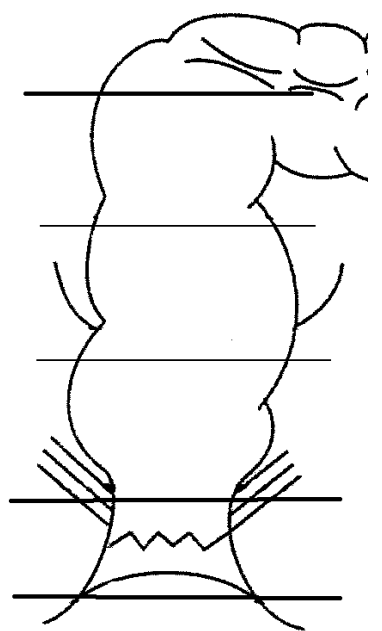
- *Appendix 3*

DATA COLLECTION SHEET FOR RECTAL CANCER SURGERY WITH ICG

- Name..... M/F
- age. Case history..... BMI:.....
- Clinical diagnosis
.....
- Clinical obstruction: NO/YES
- Neoadjuvant therapy performed: NO/YES
- 1. Clinical and imaging response. 2. No response. 3. Complete pathological response. 4. Regression

(Downsizing).....
.....

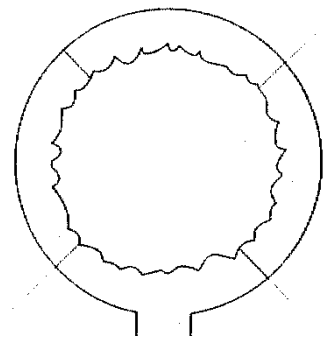
- Other concomitant/past neoplasms: NO/YES
Specify.....
- Preoperative CT or PET-CT: NO/YES
- Genetic predisposition: NO/YES
- Serum albumin:..... ASA: Hypertension: NO/YES Diabetes:
NO/YES Smoking: NO/YES
- Tumor markers:.....
- Type of operation:.....
- Total mesorectal excision: NO/YES
- Partial mesorectal excision: NO/YES
- High ligation of the vessel: NO/YES
- Splenic flexure mobilization: NO/YES
- Transanal drain: NO/YES
- Anastomosis: with a stapler / by hand
- Type of anastomosis:.....
- Stoma: NO/YES
- **Immunofluorescence data:**
- Time until the first appearance of fluorescence:
- -Angiography at the beginning:.....
- -Perfusion angiography:.....
- -Anastomosis:.....



- Change of anastomosis location: NO/YES
Direction:.....
- Materials sent:

Tumor localization/location:

- 1. Upper third of the rectum 2. Middle third of the rectum 3. Lower third of the rectum
- 4. Anal canal 5. Anus and outer skin
- A. Anterior wall B. Posterior wall C. Left wall D. Right wall
- E. Circumferential location
- I. Above the pelvic peritoneum II. Under the pelvic peritoneum III. At the level of the peritoneal fold



Macroscopic study of the fresh specimen:

- *Tumor*: dimensions...../.....cm (надлъжен/напречен)
- Macroscopic appearance: 1.superficial 2.polypoid 3. ulcerous 4. Infiltrating ulcer 5. Diffusely infiltrating 6.unclassified
- Infiltration through the wall: NO/YES
- 1. Serosa 2. Perirectal fascia 3. Adjacent organs or structures:.....
- Distance: to the proximal resection line.....cm ; to the distal resection line.....cm;
- to the anocutaneous line: 1.rectal distance.....cm 2. Fresh specimen.....cm
- Assessment of the quality of the mesorectal excision: 1.High; 2. Average; 3. Poor.....
- TNM: cT...N...M... pT...N...M...
- Number of lymph nodes:.....
- Infiltration of the resection lines: 1. Distal:.....

- 2.
- Proximal:.....
- Distance from the tumor to the circumferential boundary:.....mm
- Malignancy grade: G1 G2 G3
- Invasion of blood vessels: NO/YES
- Invasion of lymph vessels: NO/YES
- Perineural invasion: NO/YES
- Anastomotic leak: NO/YES

Date:.....

Surgeon:

VII. SCIENTIFIC ANNOUNCEMENTS AND PUBLICATIONS RELATED TO THE DISSERTATION THESIS

1. **Yotsov TI**, Karamanliev MP, Maslyankov SI, Dimitrov DD. Review on Anastomotic Leak Rate after ICG Angiography during Minimally Invasive Colorectal Surgery. *J Biomed Clin Res.* 2021;14(2):124–30.
2. **Yotsov T**, Karamanliev M, Maslyankov S, Iliev S, Ramadanov N, Dimitrov D. Mesenteric Vascular Evaluation with Pre-operative Multidetector Computed Tomographic Angiography and Intraoperative Indocyanine Green Angiography to Reduce Anastomotic Leaks after Minimally Invasive Surgery for Colorectal Cancer. *J Soc Laparoendosc Surg.* 2022;26(3) IF – 1.724
3. Karamanliev MP, **Yotsov TI**, Dimitrov DD. Complications in Transanal Total Mesorectal Excision (TATME) – Early Experience. *J Biomed Clin Res.* 2022;15(2):130–4

