“OVIDIUS” UNIVERSITY OF CONSTANȚA
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Thesis for doctoral degree
ABSTRACT

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The influence of Diabetes Mellitus as a risk factor following Colorectal Cancer surgery

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Acknowledgments,

This thesis could not have been possible without the guidance and help of my supervisor, Prof. Dr. Eugen Dumitru, who provided me with support and encouragement throughout the entire process. I would also like to give special thanks to my mentor, Dr. R.C. Popescu, for the altruistic and constant support throughout my training.

Special thanks to the Research Center of development for the morphological and genetic study in malignant pathology of Constanța, for facilitating this study.

Last but not least to my family who encouraged and supported me throughout my years of study; to my husband, who motivated me throughout my development, gave me strength to follow my dreams, to do everything with passion, desire and strength.

"Nihil sine Deo"

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Keywords: colorectal cancer, diabetes mellitus, complications, laparoscopic surgery, overall survival, MSI, BRAF, *TCF7L2*, *CASC8*, *GREM1*, rs7903146, rs6983267, rs16969681

INTRODUCTION

Colorectal cancer and diabetes mellitus are common pathologies, especially in developing or developed countries, with increasing prevalence, with important socio-economic and emotional impact. The two pathologies are associated through common risk factors, but also from a genetic point of view. Romania is a developing country, and the morbidity and mortality for both colorectal cancer and diabetes mellitus are high and continue to increase from year to year. Screening programs dedicated to these pathologies at the global level have demonstrated their benefits, but in our country there is a governmental deficiency for their implementation.

The diagnosis of cancer and even colorectal cancer is constantly increasing, even at a young age, and not infrequently in advanced stages. Oncology patient follow-up, addressability to the oncologist, and treatment were also a problem in accurately establishing the survival rate. The existence of tumor board groups to establish the management of each case according to the particularity of each patient is in a process of development and adaptation.

The existence of associated pathologies in an oncological patient makes his therapy different and should be individualized. The association of diabetes mellitus in an oncological patient represents a disadvantage for oncological therapy, for the perioperative period and for late postoperative healing. Maintaining a state of normoglycemia is difficult to manage even without the existence of cancer, but in the presence of an oncological pathology, attention must be channeled to the patient as a whole, without neglecting the associated comorbidities in clinical practice.

To avoid the important global impact of these pathologies, more should be invested in prevention; starting with individual education, recognizing symptoms, performing specific tests regardless of age when symptoms appear or when there is a genetic predisposition, establishing new screening protocols for patients with certain associated comorbidities, for those following certain chronic treatments

that could favor the development of a neoplasia and for those who associate an unhealthy lifestyle.

This PhD thesis aims to highlight the relationship between type II diabetes mellitus and colorectal cancer, to outline the impact of diabetes in patients following colorectal cancer surgery, and to prove the existence of a genetic link between the two pathologies. This thesis is structured in two parts and each part is organized by chapters. The table of contents, the numbering of Tables and Figures, as well as the references in this summary, keep the original order from the doctoral thesis.

STATE OF THE ART

The first part, the general part, includes III main chapters:

Chapter I, "Colorectal cancer" presents a review of the literature regarding current events about colorectal cancer, notions of epidemiology and etiology, pathological anatomy data, information on microsatellite instability. Colon and rectal cancer has currently become a major public health problem, with a number of approximately 2 million new cases globally (1 931 590 in 2020), the incidence being in third place in the world, after breast cancer and lung cancer, and as prevalence and mortality being the second most common neoplastic cause (1). About 2/3 of these cases are colon cancers and only one third are rectal cancers (2). In Europe, the situation of the frequency of cases is similar to that at the global level, but in Romania, colorectal cancer (CRC) has the highest incidence of all types of cancer, instead it is the 2nd cancer in terms of prevalence or mortality (1). The chapter continues to present the issue of colorectal cancer and focuses on risk factors, methods of screening and diagnosis, prognosis and treatment; on studies describing survival rates, type of treatment.

Chapter II, "Diabetes mellitus" provides information on diabetes, epidemiological and etiopathogenic characteristics, socio-demographic characteristics, risk factors such as obesity and the impact of obesity on diabetes. The chapter continues with information on the

pathophysiology and genetics of diabetes, screening methods, diagnosis and treatment.

Chapter III, "Aspects of the relationship between diabetes mellitus and colorectal cancer" presents aspects from genetic point of view and the common risk factors of the two pathologies; current and controversial aspects, recent studies, research of the human genome and individual alleles for each pathology, as well as the common ones, are also presented. The much-studied risk factor in recent decades for both diabetes and colorectal cancer, represented by the increase in body mass index. CRC and DM are two pathologies with an increasing incidence, frequently diagnosed in the same individual (291). Over time, studies have shown a predisposition to develop certain types of cancer more frequently in diabetic patients, including endometrial, breast and CRC cancers (292; 293).

The study of the relationship between DM-CRC-mortality has been a hotly debated topic, and studies have revealed that DM can negatively influence cancer patient prognosis, independent of disease stage (298-300). Patients with a known history of DM and recently diagnosed with cancer had a lower survival compared to patients without DM (301; 302). For patients with CRC, the situation was studied individually for each pathology separately, and research revealed a lower overall survival in patients who associated DM and colon cancer, compared to patients who associated DM and rectal cancer, in which this aspect was less obvious (303-305).

People with DM have a higher risk of developing a neoplastic disease, the biological mechanisms supporting this aspect include: hyperglycemia, hyperinsulinemia, insulin resistance, chronic inflammation, and sex hormone dysregulation (374). The research of tumor characteristics has developed in recent years therapeutic strategies and response to treatment that have satisfied a better prognosis. 20 years ago it was estimated that at least 15% of patients have MSI in CRC. (37) Despite all the discoveries of the last decades, MSI testing is still used as a prognostic and diagnostic marker for patients with colon cancer (458). The only study in the literature regarding the association of T2D in CRC with MSI identifies a higher frequency of T2D in MSS, compared to MSI-H; the data being similar to that of this study (464).

PERSONAL CONTRIBUTION

The personal contribution of the thesis includes the importance of the studied topic, the link between diabetes and colorectal cancer, the motivation and the main objectives; structured in III main chapters. To address the purpose of the thesis, I focused on the following main objectives:

- comparative analysis of diabetic and non-diabetic patients, recently diagnosed with CRC, from a clinical and histopathological point of view, in correlation with survival (Study I);

- establishing the influence of microsatellite instability in CRC in patients with or without DM (Study II);

- exploring gene polymorphism: TCF7L2 rs7903146, CASC8 rs6983267 and GREM1 rs16969681 in patients with CRC and T2D, compared to patients with only CRC (Study III).

Chapters III-V presenting the main findings of the thesis in accordance with the main objective, each chapter following a similar presentation model that provides an introduction, objectives, the materials and methods used in the study, the presentation of the results, the discussion of the main findings and the formulation of conclusions. These chapters include eligibility criteria, study instruments and data description, analysis procedures.

STUDY I: THE CLINICAL AND HISTOPATHOLOGICAL ASPECT OF COLORECTAL CARCINOMA IN DIABETIC PATIENTS CORRELATED WITH SURVIVAL

This chapter includes a description of the population diagnosed with colorectal cancer associated with diabetes compared to non-diabetic patients, in terms of socio-demographic characteristics, clinical and paraclinical data, characteristic treatment, analysis and findings associated with observed survival in relation to manifestations clinical, histopathological result and disease stage.

Objective:

- estimating the incidence rate, clinical and metabolic characteristics of patients with CRC that associate T2D, compared to non-DM patients;

- evaluation of the main risk factors associated with the development of CRC and DM, but also their impact on the two pathologies;

- exploring the link between T2D and CRC, establishing a temporal relationship between T2D and the occurrence of CRC and evaluating the effect of antidiabetic treatment on the incidence rate of CRC and on the invasiveness of cases;

- the study of morphological parameters: anatomical-pathological form, degree of differentiation, perineural, lympho-vascular and ganglion invasion, existence of distant metastases, tumor stage;

- histopathological stage results correlated with survival.

Materials and methods

In this prospective study, carried out between January 2018 and December 2021, patients diagnosed with a malignant tumor in the colon or rectum who addressed the General Surgery Clinic of the "Sfântul Apostol Andrei" County Emergency Clinical Hospital in Constanța were included. Patient selection was performed after elective admission, chronologically, after meeting the inclusion/exclusion criteria.

Out of 482 patients hospitalized and diagnosed with CRC between January 2018 and December 2021, 174 presented themselves in the Emergency Service, and 308 benefited from elective hospitalization. Among them: 82 patients were excluded from the study (11 with a hereditary-collateral history of CRC, 17 with a personal history of neoplastic pathology, 9 with a history of DM type I, 23 with surgical resections without anastomosis, 6 patients who refused oncological treatment postoperatively that would have influenced long-term survival and 16 patients for whom postoperative follow-up was not possible) and only 226 patients were included in the study and met the inclusion criteria.

Results

Of the 226 patients diagnosed with CRC between January 2018 and December 2021 who met the study inclusion criteria, 52 (23.01%) had associated T2DM. The patients were divided into two study groups:

Group I which included 174 histo-pathologically confirmed patients with CRC, without being known to have T2DZ; and Group II, composed of patients with CRC and previously known T2DM (52 patients). Patients with T2D were operated laparoscopically in a higher number (46.2% versus 31.6%). Regarding the number of postoperative hospitalization days, patients with pre-existing T2DM had a longer hospital stay (8.04±2.52) compared to non-diabetics (6.59±1.43), with a statistically significant difference ($p < 0.001$). In the proportion of 86.2%, patients in Group I, did not have associated postoperative complications, compared to diabetic patients (51.9%). In both univariate and multivariate analysis, there was a statistically significant difference in the increased rate of complications overall ($p < 0.001$), Grade II ($p<0.001$) and IIIB ($p=0.015$, 0.009 respectively) Clavien-Dindo and reintervention rate for T2DM patients.

The laparoscopic surgical interventions performed in non-diabetic patients compared to those with T2DZ, were characterized by: earlier resumption of postoperative mobilization (2.05±0.68, compared to 2.54±0.83, $p= 0.042$) and intestinal transit (2.24±0.54, compared to 2.92±0.78, $p < 0.001$), a lower rate of postoperative complications (1.8%, respectively 25%, $p < 0.001$) and a lower number of days of hospitalization (5.47±0.72, respectively 6.67±1.97, $p < 0.001$). Diabetic patients had advanced T3/T4 tumors (76.9% and 23.1%, respectively) compared to non-diabetic patients who were diagnosed with T1/T2 tumors in a percentage of 27%. For the majority of patients in both study groups (69%: CRC, respectively 53.8%: CRC+T2D) an $LNR<0.05$ was identified. LOODS < -1.36 was recorded for 55.2% of non-diabetic patients, and LOODS between -1.36 and -0.53 was the range recorded for 51.9% of patients who had associated T2D.

Multivariate analysis revealed that diabetic patients were 1.59 times more likely to die from CRC compared to non-diabetics. With each year added to age, the odds of dying from CRC increase by 0.4% in the general population (HR: 1.598; CI: 0.705-3.623). For patients with CRC but without T2DM, the best survival is in those diagnosed and treated in early stages I and II, followed by those in advanced stages III and IV (Figure 33.B). Diabetic patients in stage IV have the lowest average survival (25.35±5.31 months, CI: 14.95- 35.75), and for stage II and III it shows a peculiarity. Diabetics diagnosed in stage II (43.05±5.47 months, CI: 32.34-53.77) have a lower survival average

compared to those diagnosed in stage III (52.01±2.64 months, CI: 46.84-57.19) (Figure 33.B).

Assessment of weight loss in the last 6 months before diagnosis showed better survival in patients with T2D who lost more than 7% of body weight compared to those who did not show a reduction in BMI (53.23 months, HR: 2.05, CI : 49.21-57.25; respectively 41.98 months, HR: 3.44, CI: 35.23-51.89) (p= 0.015) (Figure 35.A). Analysis of Kaplan-Meier survival curves for non-diabetic patients revealed an increase in survival in patients who lost more than 7% excess body weight (54.06 months, HR: 1.55, CI: 51.02-57.11; versus 53.41 months, HR: 1.4, CI: 50.68-56.14) (Figure 35.B).

The analysis of the Kaplan-Meier survival estimation curves for the patients in the study according to the surgical intervention revealed that in the patients who also associated T2DZ that those operated laparoscopically (40.19 months, HR: 3.47, CI: 33.39-47.01) have a survival better compared to those operated open (36.37 months, HR: 2.10, CI: 32.26-40.47) (Figure 36.A). In non-diabetic patients, survival was approximately the same regardless of the type of surgical intervention, with an average of 35.18 months in patients operated laparoscopically and 37.04 months in those operated classically (Figure 36.B).

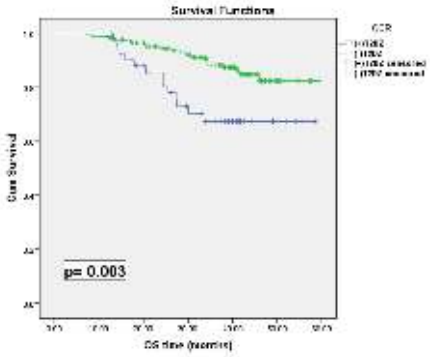

Figure 31. Kaplan-Meier overall survival analysis in CRC patients. Kaplan-Meier survival curve by T2DZ status.

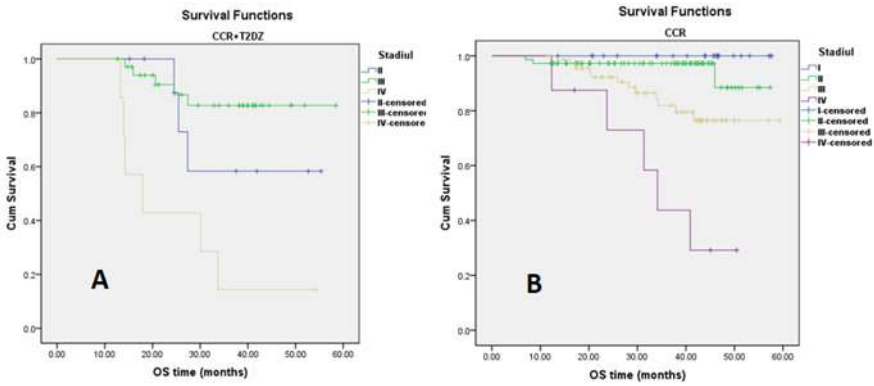

Figure 33. Kaplan-Meier survival analysis of study patients by stage. A) Patients with CRC and T2DM. B) Patients with CRC.

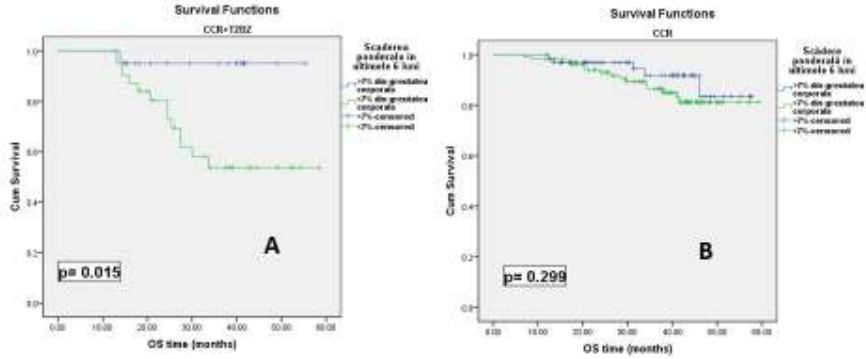

Figure 35. Kaplan-Meier survival analysis of study patients according to weight loss in the last 6 months before diagnosis. A) Patients with CRC and T2DM. B) Patients with CRC.

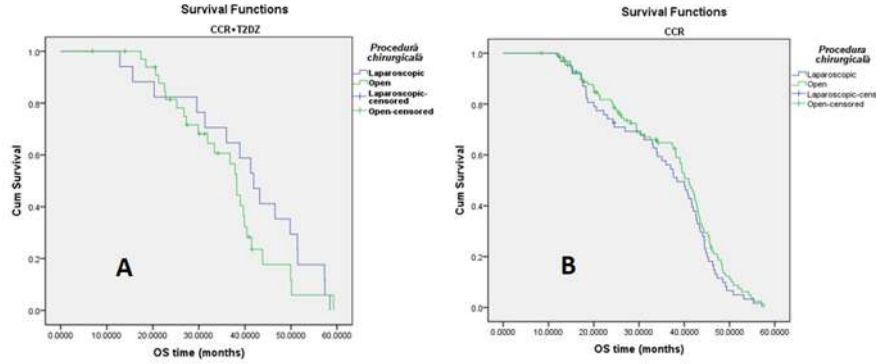

Figure 36. Kaplan-Meier survival analysis of study patients by type of surgery. A) Patients with CRC and T2DM. B) Patients with CRC

Conclusions

1. In this study, patients with colorectal cancer associating type II diabetes present a moderately-high frequency (23.01%).

2. The risk factors associated with colorectal cancer were diabetes, obesity, age, tobacco and alcohol consumption, high-calorie diet, lack of physical activity, tumor stage; risk factors negatively influencing long-term survival.

3. Losing body weight in the last months after diagnosis, by more than 7%, leads to an increase in long-term survival in patients with diabetes.

4. Diabetic patients were frequently diagnosed in advanced stages, and non-diabetic patients in a significant percentage were diagnosed in early stages.

5. The laparoscopic approach is recommended for patients with colon or rectal cancer and especially those with type II diabetes as it lowers the complication rate and increases long-term survival.

6. Mortality is higher in patients who associate colorectal cancer and diabetes compared to non-diabetic patients.

STUDY II: MICROSATELLITE INSTABILITY IN PATIENTS WITH COLORECTAL CANCER AND DIABETES MELLITUS

This chapter provides results and discussion of microsatellite instability in colorectal cancer in diabetic versus non-diabetic patients, associated with demographic characteristics, clinical, paraclinical data, disease stage, and appropriate treatment.

Objective:

- Evaluation of MSI frequency in colon cancer in diabetic and non-diabetic patients;
- Establishing demographic data and clinical-pathological and metabolic characteristics of patients according to MSI status in colon cancer;
- Identification of the frequency of BRAF mutations according to MSI in colon cancer;
- Long-term survival of colon cancer patients according to MSI status.

Materials and methods

This study is a prospective one, which included electively hospitalized patients with colon cancer in order to practice the surgical sanction, between January and December 2019. The study was carried out in the General Surgery Clinic in collaboration with the Department of Pathological Anatomy of the County Clinical Hospital of Emergency "St. Andrew the Apostle" in Constanța and with the Research - Development Center for Morphological and Genetic Study in Malignant Pathology of the "Ovidius" University in Constanța. The inclusion of patients in the study was carried out based on inclusion/exclusion criteria, in the order of admission; out of 163 patients with colon cancer, only 129 met the inclusion criteria. According to MSI status, patients were divided into 3 study groups: MSS (n=108), MSI-H (n=15) and MSI-L (n=6).

Results

Microsatellite stability was identified in 108 cases (83.7%), and microsatellite instability was identified for 21 patients (16.3%). Statistically significant differences were observed between the MSS and MSI-H groups according to gender (p= 0.010), BMI (p=0.020), T2DM (p= 0.010) and HTN (p<0.001). There were no statistical differences regarding age, background, smoking status or alcohol consumption. The frequency of T2DZ association was higher in MSS patients (60.1%), compared to MSI-L (33.3%).

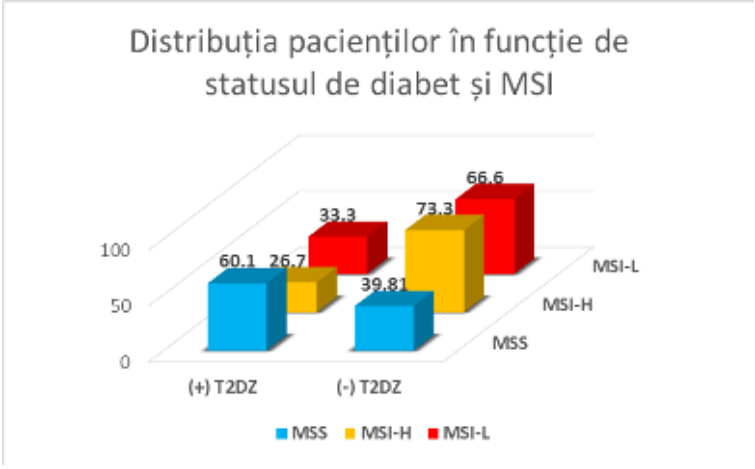

Figure 38. Distribution of cases according to T2DZ and MSI status

A frequent association was identified between patients with T2DZ and MSS (60.1%). MSI-H and MSI-L was significantly more frequent in non-diabetic patients (73.3% and 66.6%, respectively). Comparing study groups, the highest frequency of T2D patients is associated with MSS, followed by MSI-L and MSI-H.

Postoperative TNM classification of the tumors of study patients did not detect distant metastases and tumors in situ, regardless of MSI status. MSI-H tumors were predominantly pT2 (73.3%) ($p<0.001$), and MSS tumors were predominantly pT3 (53.7%) ($p=0.010$). The majority of MSI-H (53.3%) and MSS (62%) patients did not present nodal invasion, and regarding lymph node involvement there were no statistical differences between the two study groups. There were statistically significant differences between the two groups regarding lymphatic ($p<0.001$), vascular ($p<0.001$) and perineural ($p<0.001$) invasion.

Regarding the disease stage, there were significant statistical differences between the two groups regarding stage I ($p<0.001$) and III ($p<0.001$). The most frequent MSI-H cases were associated with stage I disease (66.6%), and for MSS with stage III (47.2%). Statistically significant differences also existed for the histological grade G2 ($p<0.001$) and G3 ($p<0.001$), but not for G1 ($p=0.590$). MSS tumors were mostly G2 (68.5%), and MSI-H tumors: G3 (60%).

In the case of the existence of BRAF mutations, 18 cases (13.9%) of the 129 in the study were identified, distributed as follows: 3 cases in MSS, 11 cases in MSI-H and 4 cases in MSI-L. Of the mutations studied (codon 600 and 601: V600A, V600D, V600E, V600G, V600K, V600M, V600R, K601E), only V600E was present. BRAF-mutated tumors were significantly associated with MSI-H (97.2%) and MSI-L (66.6%), in contrast to MSS tumors (2.8%). There are statistical differences between the two groups in terms of gender ($p= 0.010$). T2DZ was more strongly associated with non-mutant BRAF MSI (20%) versus mutant BRAF MSI (6.7%). Mutant MSI BRAF was associated with sixth decade of age (60.1±9.2), female sex (86.7%), normal weight (19.2±1.11), and 40% of patients being tobacco users and 26.7 potters.

From the group of patients with a negative MMPR, the most representative are MSI-L (33.3%), followed by MSI-H (14.3%) and MSS (10.2%). The majority of MSI-H patients had two negative MMPR (80%), and from the MSI-L group they had positive MMPR (66.7%). The MSS group was best represented in the MMPR positive category. The pattern with three or all negative MMPRs was not identified in any case.

Kaplan-Meier survival curve analysis indicates statistically significantly longer mean survival in patients with MSI tumors (35.11 months) compared to MSS (32.92 months) (p=0.046).

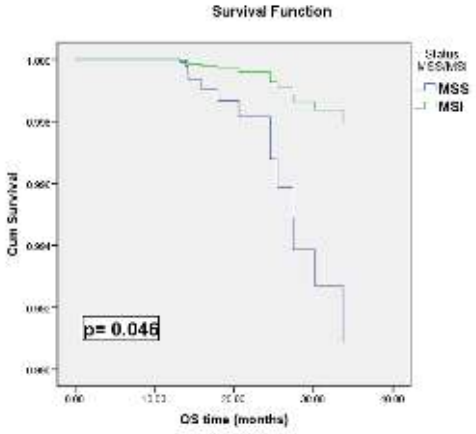

Figure 44. Kaplan-Meier overall survival analysis in colon cancer patients. Kaplan-Meier survival curve by MSI/MSS status.

Conclusions:

1. MSI frequency was recorded as 16.9%: 11.6% - MSI-H and 4.7% MSI-L.
2. Type II diabetes is associated with MSS tumors.
3. Patients with MSI-H tumors are associated with advanced age, female sex, frequent location in the ascending colon, normal weight, poorly differentiated phenotype, invasion of the own muscle and early stage of the disease.
4. BRAF mutations in colon cancer were significantly associated with MSI-H and female sex, with a frequency of 13.9%.
5. Long-term survival of MSI-H patients is significantly higher compared to patients whose tumors are classified as MSS.

3. STUDY III: ASSOCIATION OF TCF7L2, CASC8 AND GREM1 POLYMORPHISMS IN PATIENTS WITH COLORECTAL CANCER AND TYPE II DIABETES MELLITUS

Chapter V presents the results of a larger study, regarding the genetic link between type II diabetes and colorectal cancer, studying TCF7L2 rs7903146, CASC8 rs6983267 and GREM1 rs16969681 genes in patients diagnosed only with colorectal cancer, in patients with colorectal cancer and diabetes and in a control group, without associated comorbidities.

Objectives:

- Evaluation of the association of polymorphism rs7903146, rs6983267 and rs16969681 with CRC in non-diabetic patients;

- Evaluation of the association of rs7903146, rs6983267 and rs16969681 polymorphisms with CRC in T2D patients;

- Evaluation of the effect of T2DZ on patients operated on for colon or rectal cancer.

Materials and methods:

The study was a prospective, case-control type that included patients hospitalized in the General Surgery Clinic I during September 2020-2021 and was carried out in collaboration with the University's Research - Development Center for Morphological and Genetic Study in Malignant Pathology" Ovidius" from Constanța through the Grant "TCF7L2 polymorphism as a mechanism of association of diabetes with colorectal cancer". Patients were divided into 3 groups (Figure 45). The PCR method was used to identify SNPs of: TCF7L2 (rs7903146, C/T), CASC8 (rs6983267, G/T) and GREM1 (rs1696981, C/T).

Results

Statistically significant differences were recorded between the two groups for BMI ($p=0.036$), tobacco consumption ($p=0.049$), regarding the biological values of blood glucose ($p<0.001$), HbA1c ($p<0.001$), and renal tests: urea ($p=0.018$) and creatinine ($p=0.029$). Patients who associated T2D and CRC had an average BMI classified as obese (30.75 kg/m²), compared to the control group in which the average BMI classified the patients as overweight (26.31 kg/m²).

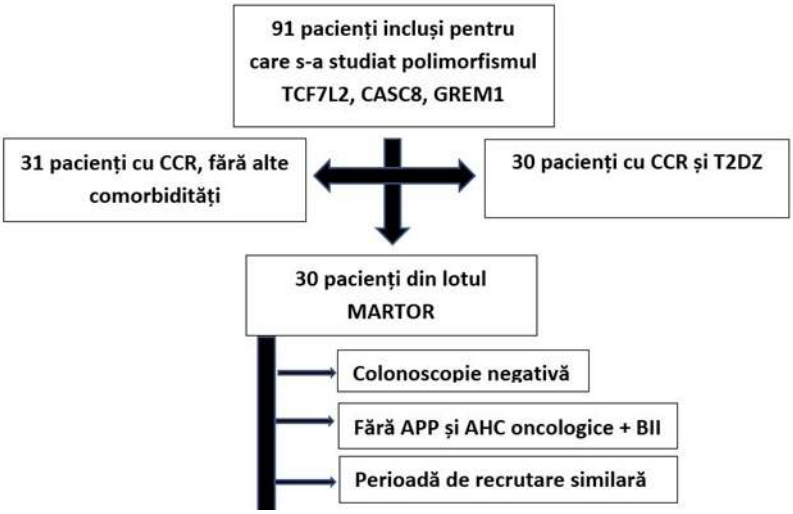

Figure 45. Processing of the recorded data of the patients from the study during September 2020-2021 in the Emergency County Clinical Hospital "Sfântul Apostol Andrei" Constanța

The time elapsed between the diagnosis of T2DM and that of CRC was between 1 and 14 years, with a mean of 7.07 ± 3.903. The mean values of tumor markers CEA and CA19-9 are above the normal limit (50.51 ng/ml and 43.15 U/ml). The mean value of TAs and TAd in patients with CRC and T2D was significantly higher compared to patients in the control group.

In the control group, the genotype distribution for the selected SNPs was consistent with the Hardy-Weinberg law with a significance level of 0.050. Univariate analysis revealed a significant association of CC+CT/TT genotypes, with risk of CRC+T2DZ for rs7903146 of TCF7L2 (p=0.003) and rs16969681 of GREM1 (p=0.009); but also of the GG+GT/TT genotypes of CASC8 rs6983267 (p=0.026). Following multivariate analysis, a statistically significant association was maintained only for TCF7L2 (p=0.021). In the case of TCF7L2, the T allele was more frequent in patients with T2D and CRC (61.7%) compared to control patients (21.7%) and could significantly increase the risk of CRC and T2D compared to the C allele (p<0.001 , OR: 3.865, CI: 1.743–8.567). This aspect is not identified for rs16969681, where the T allele was less frequent both in diabetic patients with CRC (38.3%) and in the control group (25%), without having statistical

significance (p=0.116); thus the existence of a T allele does not increase the susceptibility for CRC and T2DZ.

Table 24. Uni-/multivariate analysis of diabetic patients with CRC and the control group according to SNP

SNP	Univariate analysis			Multivariate analysis	
	CRC+T2DM (n=30)	Controls (n=30)	p value	OR [95%CI]	p value
rs7903146					
CC+CT	21 (70)	29 (96.7)	-	-	-
TT	9 (30)	1 (3.3)	*0.003*	0.080 [0.009-0.685]	*0.021*
rs6983267					
GG+GT	21 (70)	25 (83.3)	-	-	-
TT	9 (30)	5 (16.7)	*0.026*	2.143 [0.622-7.387]	0.227
rs16969681					
CC+CT	22 (73.3)	26 (86.7)	-	-	-
TT	8 (26.7)	4 (13.3)	*0.009*	2.364 [0.627-8.917]	0.204

Variables are expressed as number of cases, with percentages in parentheses. Italic values indicate statistical significance ($p < 0.050$). SNP, Single Nucleotide Polymorphism; CRC, Colorectal Cancer; T2DZ, Diabetes Mellitus Type II; OR, odds ratio; CI, Confidence interval.

Genotype distribution analysis for study groups (CCR/Control) of selected SNPs (rs7903146, rs6983267, rs16969681) identifies statistically significant differences in genotype distribution. The results of the analysis of the dominant and recessive model of the comparatively selected SNPs for the studied groups, no significant correlation was identified in the dominant models as having a risk of developing CRC, when comparing the homozygous genotype (TT) with the genotypes CT+CC and GT+GG . When comparing the TT+CT genotypes, with the CC genotype of rs7903146, a statistically significant association was revealed (p=0.036, OR: 1.600, CI: 0.580-

4.414) for the recessive models. There was no statistically significant correlation with CRC risk in the recessive patterns of rs6983267 (p=0.510) and rs16969681 (p=525).
Comparing normal-weight patients with overweight and obese patients, there is a significant increase in the risk of developing CRC in patients with BMI ≥ 25kg/m², compared to those in the control group (p= 0.037). No statistically significant correlation with BMI was identified for rs6983267 and rs16969681.

Table 29. Genotype distribution and recessive pattern analysis of rs7903146, rs6983267, rs16969681 in CRC patients compared to controls

Genotip	CRC (n=31)	Controls (n=30)	OR [95%CI]	p-value
rs7903146				
TT+CT (%)	16 (51.6)	12 (40)	Referință	-
CC (%)	15 (48.4)	18 (60)	1.600 [0.580-4.414]	*0.036*
rs6983267				
TT+GT (%)	24 (77.4)	21 (70)	Referință	-
GG (%)	7 (22.6)	9 (30)	1.469 [0.466-4.633]	0.510
rs16969681				
TT+CT (%)	9 (29)	11 (39.6)	Referință	-
CC (%)	22 (71)	19 (63.3)	0.707 [0.241-2.068]	0.525

Variables are expressed as number of cases, with percentages in parentheses. Italic values indicate statistical significance (p < 0.050). CRC, Colorectal Cancer; OR, odds ratio; CI, Confidence interval; TCF7L2, Transcription factor 7-like 2; CASC8, Cancer Susceptibility 8; GREM1, Gremlin 1.

Statistically significant differences between the two groups (diabetic/non-diabetic) were identified regarding BMI (p= 0.023), blood glucose values (p<0.001), HbA1c (p< 0.001) and tumor marker CA19-9 (p= 0.015). There were statistically significant differences between the two groups regarding the duration of surgery (p= 0.013), postoperative complications (p= 0.046) and the number of postoperative hospital days (p= 0.042). Laparoscopic surgical interventions were less frequent in patients with DM (26.7%) compared

to non-diabetics (35.5%). Only one T2D patient required reoperation. There were no postoperative deaths in the first month and no readmissions.

Table 32. Perioperative characteristics of CRC patients according to T2DM

Variables	**CRC (n=61)**		**OR [95% CI]**	**p-value**
	(+) T2DM (n=30)	**(-) T2DM (n=31)**		
Surgery tipe* Laparoscopic Open	 8 (26.7) 22 (73.3)	 11 (35.5) 20 (64.5)	 Referință 0.488 [0.120-1.833]	 - 0.457
Time of surgery (min)	191.8±20.78	196.1±25.67	0.048 [0.015-0.964]	*0.013*
Complications*	9 (30)	3 (9.7)	0.250 [0.060-1.038]	*0.046*
Reinterventions	1 (3.3)	0	0.967 [0.905-1.033]	0.305
Postoperatyve hospitalization	7.03±1.21	6.13±0.88	-	*0.042*
30 dazs postoperative mortality	0	0	-	NS
Readmission	0	0	-	NS

Variabilele sunt exprimate ca medie ± SD (Deviația Standard), dacă nu se indică altfel. *Număr de cazuri, cu procente între paranteze. Valorile italice indică semnificația statistică ($p < 0.050$). CCR, Cancer colorectal; T2DZ, Diabet Zaharat Tip II; SAU, raportul de cote; po, postoperator.

Postoperative complications of CRC patients were calculated Clavien-Dindo classification, Minor complications (Grade I: 3.2%, Grade II: 3.2%, Grade IIIA: 3.2%) were common in non-diabetic patients, compared major complications (≥ Grade IIIB= 0). Non-diabetic patients had minor and major complications recorded, the highest frequency being Grade II (16.7%), the major complications totaling 3.3%, and the highest class of complications being IIIB. Clavien-Dindo grade IV and V was not identified in any patient. Postoperative complications commonly encountered in study patients are illustrated comparatively for diabetic and non-diabetic patients in Figure 56.

Diabetic patients associated advanced tumors in significant T3/T4 proportions (70%), and non-diabetics had incipient tumors with invasion up to the level of the own muscle in a proportion of 35.5%. Secondary determinations were registered in 10% of cases of patients with T2DM, compared to patients without DM: 6.5%. Following the univariate analysis, differences between the two categories (open/laparoscopic) were highlighted, with statistical significance for laparoscopic interventions: postoperative ileus (p= 0.008) and the duration of the surgical intervention (p= 0.002). The duration of the surgical intervention maintains its statistical significance also in the multivariate analysis (p= 0.024), to which is added an important association of laparoscopic interventions with: a Grade ≤3 postoperative complications (p= 0.040), with the absence of postoperative infection (p= 0.040).

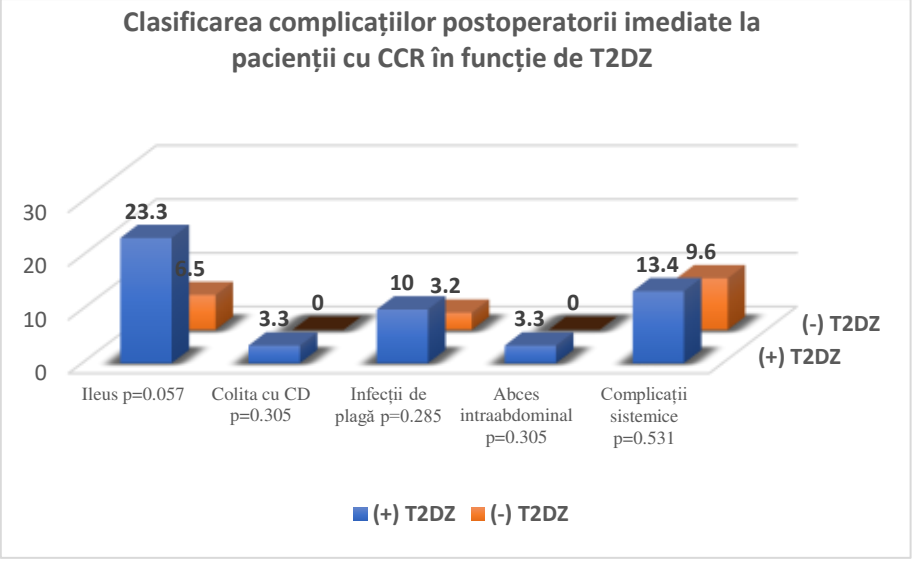

Figure 56. Classification of immediate postoperative complications in CRC patients according to T2DM

Conclusions:

1. Colorectal cancer development shows an association with the rs7903146 polymorphism of TCF7L2 with low statistical significance but increased risk for the homozygous (TT) genotype.

2. There is no association between the polymorphism rs6983267 of CASC8 and rs16969681 of GREM1 with colorectal cancer.

3. A statistically significant association was identified between rs7903146, rs6983267, rs16969681 and patients with type II diabetes and colon or rectal cancer.

4. Type II diabetes negatively influences the rate of postoperative complications after surgery for colon or rectal cancer, with increased hospitalization time.

5. Laparoscopic surgery, by improving postoperative results, benefits patients with type II diabetes.

7. ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

This paper investigates the link between type II diabetes and colorectal cancer from a demographic, clinical, biological, histo-pathological, surgical management, and genetic point of view. The original elements of the thesis consist in the cumulative results obtained in each of the three completed studies.

The first study, **"The clinical and histopathological aspect of colorectal carcinomas in diabetic patients correlated with survival"** is the only large study in Romania that addressed the existence of a link between type II diabetes mellitus and colorectal cancer, the impact of diabetes in the perioperative period and long-term survival of these patients. The evaluation of the characteristics of these patients determines the establishment of new screening, diagnostic and therapeutic protocols in order to increase the quality of life and the duration of survival.

The second study, entitled "**Microsatellite instability in patients with colon cancer diabetes mellius"**, gives the approach to the status of microsatellite instability in patients with colorectal cancer, the demographic, clinical-biological and morpho-pathological characteristics of these patients, with the comparative approach of diabetic patients and non-diabetics; being among the few studies in the international specialized literature and the only one in Romania that studies the long-term survival of MSI/MSS patients, but also the association of T2DZ with MSI.

The originality of study III, "Polymorphism of TCF7L2, CASC8 and GREM1 in patients with colorectal cancer and type II diabetes mellitus" is conferred by the study of polymorphism rs6983267 of CASC8 and rs16969681 of GREM1 in patients with colorectal cancer with type II diabetes mellitus and without diabetes, compared to a control group, being the first study in the field that addressed a separate category of patients.

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