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THE IMPORTANCE OF LYMPH NODE AND PERINEURAL INVASION IN THE EVOLUTION OF COLON CANCER

SUMMARY OF THE DOCTORAL THESIS

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Content

List of papers published in ISI indexed journals:

1. Early perianastomotic tumoral recurrence in a patient with sigmoid colon adenocarcinoma with perineural and lymphovascular invasion

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2. Lymphovascular invasion in the evolution of colon cancer: a single center experience and analysis of the specialized literature

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3. Perineural invasion in the evolution of colon cancer: a single center experience and analysis of the specialized literature

Emil-Marian Popescu; Dan-Alexandru Popescu; Daniel Alin Cristian; Traean Burcoș; Diana-Andreea Popescu, Chirurgia (Bucur). 2025 March, Online Ahead of Print, ISI indexed journal with impact factor 0.8, http://dx.doi.org/10.21614/chirurgia.3114 (original research article made from chapter 6).

Introduction

At the beginning of this study, in 2018, the incidence of all neoplasms in Europe was around 23%, and the number of deaths was slightly above 20%. Cancer was the second leading cause of morbidity and mortality in Europe. Currently, according to data provided by Globocan in 2022, colorectal neoplasm ranks second in Europe, with an incidence of 12% in 2022, first place being occupied by breast neoplasm with 12.5%, and third and fourth place being bronchopulmonary neoplasm with 10.8% and prostate neoplasm with 10.6%, respectively (Ferlay J, 2024). Information on cancer mortality at European level provided by the same agency places colorectal cancer in the same second place with 12.5%, first place being occupied by bronchopulmonary cancer with 18.9%, and third place being occupied by breast cancer with 7.3% (Ferlay J, 2024).

At the national level, colorectal cancer is the most common neoplasia, followed by breast cancer and lung cancer, with a slightly stationary incidence in recent years, reaching a value of 13,541 new cases. (Ferlay J, 2024). Of all the neoplasias detected in our country, the incidence of colorectal neoplasm represents a reported percentage of 12.9% in 2022, compared to 13.3% in 2018.(Ferlay J, 2024). Since 2020, with an incidence of 13.1%, colorectal cancer has occupied the first position, which highlighted a significant increase in the number of cases reported in 2018, 1.3% above the European average. The death rate from colorectal cancer was estimated at 13.1% in 2022, a slight increase of 0.4 percentage points compared to the estimated value for 2018.

Frequently diagnosed in advanced stages, colorectal cancer is a disabling disease that sometimes irreversibly affects the patient's quality of life. Due to the significant costs generated by the late diagnosis of the disease, costs grafted on by increased morbidity and mortality, colorectal neoplasm has benefited over time from numerous technological advances, starting from screening tools, very useful for detecting the disease in its early, incipient phase, to neoadjuvant and adjuvant therapy tools, in case the initial staging requires such preoperative and/or postoperative treatment. (Bhimani N, 2022). Surgical treatment has benefited from the contribution of minimally invasive surgery, which is currently enjoying a fulminant rise, both through the laparoscopic approach and through the robotic or combined approach. The possibility of performing a complex surgical intervention with the intention of oncological radicality in a

minimally invasive manner has allowed a shorter hospitalization period, a lower rate of immediate or late postoperative complications compared to classic, open surgery, a faster social reintegration, without having side effects on the subsequent evolution of the disease. (John C. Taylor, 2024). Numerous studies conducted and published in the literature have shown that the recurrence rate and mortality from colorectal cancer are not affected by the type of surgical approach chosen and practiced. Currently, guidelines recommend the minimally invasive approach in colorectal surgery as the first choice, when the situation allows it, as it has proven to be superior to open surgery, an aspect discussed previously. (Zeng WG, 2014).

The need for primary prevention in neoplastic pathology is evident and numerous published studies emphasize the importance of identifying risk factors, thus establishing the target population, prone to developing a certain neoplasia throughout life, thus screening means demonstrate their efficiency and justify their costs, but they are not widely available in underdeveloped and underfunded health systems. This aspect makes early diagnosis and the establishment of prompt and adequate treatment difficult, compared to the pretherapeutic staging necessary for the oncological patient. The difficult access to screening means, combined with the poor medical education of the general population, represent the most important factors that together contribute to a mortality rate well above the European average from colorectal cancer. The interest shown by researchers on a large scale in risk factors, diagnostic methods and treatment options, has materialized in the establishment of standardized diagnostic and therapeutic management protocols.

The motivation for choosing the topic is the need to implement a national screening program for the early detection of neoplastic processes with colorectal involvement, as well as the need to create a national database containing data on all patients diagnosed with colorectal cancer. The database must contain the complete patient file that includes data related to the family history, personal pathological history, investigations performed, treatment followed, as well as its evolution over time.

I. CURRENT STATE OF KNOWLEDGE

1. Risk factors in colorectal cancer

Despite the technological and logistical advances recorded in recent decades, both at the surgical level, through the introduction of minimally invasive laparoscopic and robotic techniques and mechanical sutures, and at the oncological or radiotherapy level, the average 5-year survival in colorectal neoplasm slightly exceeds 60% in countries considered developed, with a well-implemented national screening program that is widely accessible to the general population. (Brouwer NPM, 2018). In the rest of the countries, the average survival is considerably lower, around 40% (Rawla P, 2019). The likelihood of an individual developing colorectal cancer over their lifetime differs depending on their gender. A ratio of 1 in 17 is valid for males, who are more prone to developing the disease, and a ratio of 1 in 18 for females (Amersi F, 2005).

Studies in the specialized literature have proven that a good knowledge of the risk factors implicated in the occurrence of colorectal cancer is essential in identifying the population at risk, thus the usefulness of screening tools and prevention measures refers primarily to the targeted population and then to the general population (Pop, 2009).

The main risk factors implicated in the occurrence of colorectal cancer will be detailed below:

1.1. Patient's age

The patient's age, in close correlation with the physiological aging process, undoubtedly represents a factor that can lead to an increased risk of neoplastic diseases. Colorectal neoplasms are no exception to this rule, over 85%-90% of newly diagnosed cases are found in patients over 55 years of age, without being able to exclude the possibility of the disease occurring at a much younger age (Roshandel G, 2024). In the literature, the average age at the time of diagnosis of the disease is around 60 years old, which is true for both sexes, with a median age of 70 years for males and a median age of 72 years for females (Glover M, 2019). The worrying aspect regarding the continuously increasing incidence of colorectal cancer is given by the increasingly younger age at the time of diagnosis of the patient. It is estimated that a percentage of almost 15% of all

colorectal cancer patients is represented by patients under the age of 50 (Chen FW, 2017). This explains the need to implement and perform screening measures much earlier than the initial recommendations, starting at age 45 (40 years for people at risk) periodically until age 75 or until life expectancy is less than 10 years, with a variable frequency depending on the particularities of each individual (Anon., fără an). People between the ages of 76 and 85 may benefit from screening, based on their previous results and personal preferences. The guidelines do not recommend screening for people over 85.

1.2. Sex

Compared to women, specialized studies have observed that men have a slightly higher risk of developing colorectal cancer, without being able to specify the cause and mechanisms underlying this finding (Roshandel G, 2024). Estrogenic hormonal activity is considered a protective factor in women of childbearing age, but hormone replacement administered over long periods of time cannot be considered as a means of protection, due to the adverse effects that may occur at the level of other organs and systems (González-Flores, 2025).

Estrogen has an antitumor role in the colorectal mucosa by binding to its beta receptor. This binding causes the activation of apoptotic mechanisms with an onco-suppressive role, the loss of this connection in the development of colorectal cancer may suggest an important role of estrogen in the progression of the disease (Caiazza F, 2015).

1.3. Subjects' race

Numerous studies have reported statistically significant differences in survival rates between different racial groups. African Americans have a higher incidence and lower survival compared to Caucasians (Amersi F, 2005). The 5-year survival data, comparing the two breeds, differ depending on the stage of the disease at the time of diagnosis. The percentage variation is between 7% in the first two stages (I and II) and 10% between the last two (stage III and stage IV) (Amersi F, 2005).

The differences noted in the studies can be attributed to the socio-economic problems of the African-American race, based on underfunded health systems, based on the lack of screening programs and the low addressability of patients to health services.

1.4. Obesity and sedentary lifestyle

Patients with a BMI greater than 30, compared to the rest of the population, are at increased risk for developing colorectal cancer (Mandic M, 2024). A similar situation is found in those with low physical activity. Hyperinsulinism due to increased insulin resistance is the hypothesis by which the researchers try to establish the causal relationship between the increased risk of colon cancer and type II diabetes (Chen, 2018). Moderate-increased physical activity has been proven in all studies to be a protective factor in the occurrence of the disease, while increased calorie intake and/or sedentary lifestyle are negative factors (Amirsasan, 2022).

1.5. Diet and alcohol consumption

Perhaps one of the most important environmental factors that can lead to the development of colorectal cancer over time is poor nutrition, both quantitatively and qualitatively (Vallis J, 2022). It is known that a diet low in fiber and high in saturated fats, red meat and/or industrially processed meat increases the risk of colon cancer through prolonged contact and the action of these products on the colorectal mucosa (Ryan-Harshman M, 2007).

Alcohol consumption is also an independent risk factor for the development of colorectal cancer, the risk being directly proportional to the total amount of alcohol consumed by the patient (V. Fedirko, 2011).

1.6. Tobacco use

The development of colorectal cancer involves a slow process, carried out over a long period of time. The appearance of colorectal cancer is translated by the transformation of the normal mucosa of the digestive tract through several intermediate stages to invasive neoplasm from premalignant lesion (dysplasia) to malignant lesion (Duan B, 2022). Most colorectal neoplasms develop through two sequences. The most common sequence is represented by sporadic malignancy of adenomatous polyps, a situation encountered in more than 70% of cases (Bujanda L, 2010).

Smoking is an independent risk factor (Gram IT, 2020). Its existence predisposes to the appearance of colorectal polyps, considered to be precancerous lesions with subsequent potential for malignant degeneration, a time-dependent process (Amitay, 2020). Another potential mechanism by which smoking may increase the risk of colorectal cancer is the alteration of the

intestinal microflora. A 2009 cohort study in the United States of America attested to a 30% increase in the incidence of colorectal cancer in active smokers (Hannan LM, 2009).

1.7. Type II diabetes mellitus

According to the data available in the literature, the incidence and prevalence of type 2 diabetes mellitus is continuously increasing. In 2019, 463 million people were reported to have diabetes mellitus, representing 9.3% of the global adult population aged 20 to 79 years. For the year 2030, researchers estimate the increase in the number of diabetic people to 578 million (10.2%), and for the year 2045, the estimates increase to 700 million, representing a percentage of 10.9% globally (Pouya Saeedi, 2019). Due to the complex interactions of the disease with all organs and systems, it has been hypothesized that type II diabetes mellitus, along with obesity, actively participates in oncogenesis processes, not only for colorectal cancer. Persistently elevated values of glycosylated hemoglobin HbA1C, determined by constant periods of hyperglycemia, lead to increased production of proinflammatory cytokines TNFα and IL6 and reactive oxygen species (Lawler T, 2023). Persistent inflammation, in close connection with chronic oxidative stress, are additional risk factors for colorectal cancer (Yu GH, 2022). Regardless of the duration of the disease, diabetes is considered a risk factor. The most affected are patients undergoing chronic treatment at home with insulin derivatives, the risk of which is twice as high compared to diabetics undergoing treatment with oral antidiabetics (Ferroni P, 2016).

1.8. Crohn's disease and RCUH

In Crohn's disease and ulcerative colitis, the risk of colorectal cancer increases directly proportionally to the extent and severity of the disease, as well as its duration, through qualitative and quantitative alteration of tissues, due to persistent inflammation, grafted by bouts of inflammation and remission, specific to these pathologies (Stidham RW, 2018). Factors that contribute to the development of colorectal cancer are represented by the onset of the inflammatory disease at a young age, the long-term evolution, the extension to the colon and the existence of colorectal cancer in the patient's family history (HJ, 2008). After a long evolution of the pathology, the risk increases cumulatively, and as a reference value for this disease, the risk is around 2% per 10 years. The risk increases directly proportional to the degree of extension of the disease at the colonic level.

1.9. Personal pathological history

The patient's personal pathological history refers to colorectal neoplasms or other types of neoplasia in the patient's personal history, as well as the irradiation history (Sawicki T, 2021). In this category we can also include the presence of intestinal polyps, where the risk of developing colorectal cancer increases exponentially with their number, an aspect dictated by the time elapsed from their appearance to the moment of diagnosis.

1.10. Patient's family history

Over 30% of colorectal malignant tumors occur in a hereditary context, thus an individual's risk of developing a colorectal neoplasm doubles in the context of having a first-degree relative (parents/brothers/sisters) diagnosed/treated for this pathology (Jasperson KW, 2010).

2. Prognostic factors in colorectal cancer

2.1. Prognostic factors closely related to the primary tumor at the time of diagnosis

The factors closely related to the primary tumor at the time of diagnosis can be summarized in factors related to the initial tumor size, its degree of invasiveness compared to adjacent tissues and the macroscopic appearance present both on colonoscopic and intraoperative visualization, the urgency of the intervention and the resection margins after the establishment of surgical treatment. Some particular aspects related to these factors will be presented in this material.

2.1.1. Tumor size

Tumor size, included in the TNM classification and staging of colorectal cancers, was initially considered a negative prognostic factor, always associated with local tumor invasion and distant dissemination. Over time, studies have demonstrated in the case of colorectal neoplasms, an exception to this rule, confirmed by the fact that tumor size did not statistically correlate with a more reserved prognosis, and the average 5-year survival was not significantly affected. Tumor size at the time of diagnosis can influence the surgical therapeutic attitude and the type of approach as well as the patient's immediate postoperative evolution, but in the long term, the evolution of these patients from a prognostic point of view is relatively similar to those initially diagnosed with less voluminous tumors (Balta AZ, 2014).

2.1.2. Macroscopic tumor invasion of the primary tumor

Macroscopic tumor invasion of neighboring organs and the peritoneal serosa represents an advanced tumor stage, consistent with TNM staging. This has been studied for a long time, especially from the oncological prognostic perspective of the evolution of these patients (MIM, 2023). A major impact was found, with a poor prognosis for patients where invasion into peritumoral structures was histopathologically confirmed and a much more severe impact in the presence of peritoneal tumor seeding, in which case, 5-year survival tends to 0% (Monahan BV, 2023).

2.1.3. Macroscopic appearance of the primary tumor

Histopathologically, a relevant aspect and a negative prognostic factor is the macroscopic appearance of the primary tumor and its postoperative resection margins (L. Ludeman, 2006). Unlike vegetative tumors, ulcerative-infiltrative tumors seem to have a more reserved oncological prognosis, represented by their increased capacity for distant dissemination and the increased risk of perforation of the affected organ, with subsequent complications of peritonitis (Otani K, 2019).

2.1.4. Tumor resection margins

A particular, controversial aspect of modern colorectal surgery is that of the oncological safety margins found on the postoperative resection specimen. It is considered sufficient to achieve resection margins of at least 5-7 cm upstream or downstream, related to the tumor location in colorectal surgery with a radical oncological goal (Ueno H, 2023). This goal is achieved to minimize the risk of locoregional tumor recurrence, especially at the level of the colonic heads remaining after the excision of the tumor piece, regardless of whether the surgical intervention ends with anastomosis or external derivation (Aarons CB, 2013).

2.1.5. The emergency nature of the surgical intervention

With a severe negative impact, it is also necessary to mention the tumor with occlusive digestive phenomena, especially in the case of vegetative, voluminous tumors, located in the right colon and transverse colon, which require prompt, emergency surgical treatment, as well as small stenosing tumors located in the left colon and rectum, prone to diastatic cecal perforation (Ziani H, 2024). Such interventions do not allow for adequate preoperative staging and eliminate the potential benefits of neoadjuvant chemotherapy treatment, with staging of the case being performed postoperatively, when the patient's condition allows it (Georgiana B. Constantin, 2020).

2.2. Histopathological prognostic factors of the primary tumor in colorectal cancer

The late discovery of colorectal neoplastic disease, in advanced stages, often implies the possibility that it has disseminated to other organs and systems. The localized disease thus becomes a systemic disease with implications and complications that vary depending on the new location(s). The dissemination process of neoplastic disease is a known process, described in the literature and evaluated in the TNM staging of colorectal neoplasms. The most known and studied dissemination routes of colorectal neoplasm are the lymphoganglionic, lymphovascular and perineural:

2.2.1. Lymph node dissemination

Histopathologically detected tumor invasion of the locoregional lymph nodes is one of the most important negative prognostic factors in the evolution of colorectal neoplasm. The absence of invasion certified on the histopathological reports of the resected pieces from a radical oncological surgical intervention (R0 resections) can be correlated with a survival prognosis of up to 90% evaluated at 5 years, this decreasing dramatically in the situation where secondary tumor determinations are confirmed in the intermediate lymph node drainage stations.

A particular, much more severe situation refers to tumor invasion of the central lymph nodes, which suggests a possible dissemination of tumor cells distant from the primary tumor. Lymph node invasion is an important parameter and undoubtedly a negative prognostic factor in the 8th TNM classification, where the anatomopathologically certified presence of tumor cells represents at least a tumor stage IIIA.

Currently, current guidelines recommend histopathological evaluation of at least 12 peritumoral lymph nodes on the resected specimen in order to be able to establish with certainty the presence or absence of secondary distant findings with lymph node involvement (Compton CC, 2004). The examination by the pathologist of as many lymph nodes as possible allows for better staging of the case, thus avoiding diagnostic errors and erroneous classification, through a false negative result in a less advanced tumor stage (Weiser, 2018). The statistically significant difference in survival rates in patients with colorectal cancer diagnosed at an advanced stage has led to the need to subclassify lymph node invasion according to the number of histopathologically confirmed invaded lymph nodes out of the total lymph nodes examined on the surgical specimen. The 8th TNM staging proposed by the AJCC subclassifies lymph node dissemination according to

the number of lymph nodes found to be invaded (Arrichiello G, 2022). Thus, 1-3 tumor-invaded lymph nodes represent the N1 category, a category subdivided into 3 subgroups (Ueno H, 2023). Tumor involvement of more than 4 lymph nodes represents category N2, a category subdivided into 2 subcategories (Weiser, 2018).

Correct staging of colorectal neoplasm can often be difficult to achieve (Puppa G, 2010). The difficulty in performing a correct staging is mainly due to the presence of micrometastasis at the lymph node level (G. Botiralieva, 2016). Microscopic disseminations are often missed in the histopathological examination, which argues for a therapeutic management that is sometimes inadequate to the case, due to its classification in a less advanced tumor stage (H, 2022). Microscopic examination sometimes does not show any suggestive features for the presence of secondary lymph node tumor determinations (Dipti M. Karamchandani, 2020). Micrometastatic invasion is considered an independent risk factor, with a major impact on the outcome of a patient with colorectal cancer (Hoorens, 2022). Its existence can influence the evolution of the oncological patient in two main directions. Firstly, it can lead to an increase in the risk of distant dissemination through the presence of isolated tumor cells not visualized microscopically at the lymph node level, and secondly, through adjuvant oncological treatment inappropriate to the existing tumor stage (Park SJ, 2008). Identifying the presence of micrometastasis involves significant increases in the costs allocated to the diagnosis and treatment of an oncological patient. Immunohistochemistry and PCR techniques are currently not widely used in routine practice, having elective indications in colorectal cancer (Omran, 2024).

2.2.2. Lymphovascular invasion (LVI)

Lymphovascular invasion (LVI) is defined by the presence of tumor cells in blood vessels or lymphatic vessels, visualized by microscopy. It is imperative to mention the technical difficulty faced by the pathologist. The detection of lymphovascular invasion is a negative prognostic factor that can influence the subsequent therapeutic decision. The presence of tumor cells at this level indicates an increased potential for distant dissemination of the primary tumor (Harris EI, 2008) (Gao, 2021). Colorectal neoplasms with positive lymphovascular invasion present an increased risk of locoregional recurrence (Wang X, 2021).

The presence of tumor invasion in small non-muscularized vessels can be represented by invasion of postcapillary lymphatics or venules, both variants representing negative prognostic

factors (Santos C, 2013). Vascular invasion in colorectal neoplasms diagnosed at an advanced stage is represented in more than 99% of cases by venous invasion. Microscopic detection of invasion on histopathologically analyzed pieces has been classified as a negative prognostic factor. In situations where the presence of vascular invasion is certified, the risk of locoregional recurrence increases exponentially, similarly, the risk of secondary determinations at a distance (Bianchi, 2021). Extramural venous invasion has a much more severe impact on disease-free interval and 5-year survival compared to intramural invasion (Knijn N, 2018).

2.2.3. Perineural invasion (PNI)

Perineural invasion (PNI) is a relatively new parameter that has received little attention in the literature until recently, with dynamics that are not fully known, with multiple molecular mechanisms involved (Qi Liu, 2022). Described in the early 1800s, the perineural dissemination pathway is of particular importance today and represents a negative prognostic factor in neoplastic processes of the prostate, pancreas, stomach, biliary tract and in neoplastic processes with colorectal locations (Chen SH, 2019) (Schouten TJ, 2024). The first definition of perineural invasion was developed by Batsakis much later, in 1985 (JG., 1985). His findings refer to microscopic invasion of the connective nerve structures that make up the nerve, which have the lowest degree of resistance. The perineural tumor invasion described by Batsakis can be directly observed by neighboring invasion or by endolymphatic dissemination (JG., 1985). Starting from his research, several theories regarding perineural tumor invasion were described in the specialized literature, with the lymphatic pathways as the main dissemination route, but these were later abandoned. Later published studies demonstrated the lack of lymphatic vessels inside the nerve sheath (Reina MA, 2000).

The way in which perineural invasion is constituted requires detailed knowledge of the basic structure and composition of the peripheral nerve sheath. The nerve is made up of 3 layers of connective tissue. Its components are represented by the epinerve, the perinerve and the endonerve.

The epinerve represents the peripheral layer of the nerve and is composed of an external layer that has in its composition areolar connective tissue and an internal layer made up of elastic fibers and collagen fibers. The main role of the epinerve is represented by the interconnection of

one or more fascicles in a single nerve (Reina MA, 2020). The epinerve contains lymphatic vessels and the epineural component of the vasa nervorum.

The perinerve is the middle layer of the nerve sheath. Its structure comprises endothelial cells arranged concentrically, in layers, delimited on each side by the basement membrane (Akert K, 1976).

The endonervous system is the inner layer of the nerve structure. This last layer is made up of intrafascicular connective tissue arranged around the nerve fibers, axons and Schwann cells. The connective tissue accompanies the existing endoneurial blood vessels, and this structure is called the blood-neural barrier (S, 2015).

Currently, the diagnosis of perineural invasion refers to the detection by microscopy during histopathological examination of tumor invasion in any of the 3 layers that make up the structure and composition of the nerve sheath: epinerve, perinerve and endonerve (Liebig C, 2009).

For perineural invasion, several scenarios have been described by which tumor cells can come into contact with nerve structures. Thus, in the current definition of perineural invasion, the contact of tumor cells can be tangential through an intimate contact of the tumor with the nerve or a contact with circumferential invasion through the inclusion of the nerve partially or completely in the tumor structure (Goswami PR, 2023). These microscopic aspects are pathognomonic and easily recognizable on histopathological examination, if the perineural invasion is distant from the primary tumor and much more difficult, if the perineural invasion is within the primary tumor. Perineural invasion is considered positive when at least 33% of the nerve circumference is affected by tumor, any value less than 33% represents focal tumor infiltration and cannot certify perineural invasion (Liebig C, 2009).

II. PERSONAL CONTRIBUTIONS

3. Working hypothesis and general objectives

The current study analyzes 241 hospitalized patients, diagnosed with colon cancer and operated on in the Surgery Clinic of Colțea Clinical Hospital. The data were obtained by analyzing the medical documents available in the clinic. The patients were staged and followed up for a short period. The evaluation of the disease-free interval and survival was not possible, as many patients were lost from the records. The age and sex of the patients are variables of the study that must be compared with the literature data. An increase in the incidence of this neoplasm was also noted in women (data reported by Globocan), so a possible certification in this group is also required. Age is an important factor in the therapeutic attitude, but it must be analyzed as a dynamic component, with a significant population impact. Tumor location is relevant, and its variability within the study requires additional analysis. This may delay the patient's presentation to the doctor through minimal symptoms (in the absence of effective screening programs) and may generate medical and/or surgical complications that may transform an elective multidisciplinary approach into an emergency indication with a possible palliative goal. The secondary anemic syndrome generated has a complex etiology and requires particular management. The histopathological type and degree of differentiation are essential to this study and require correlations with the presence or absence of perineural and/or lymphovascular invasion, as well as with tumor location.

The aim of the study is to analyze the data obtained to assess the importance of perineural and lymphovascular invasion as well as lymph node invasion in patients with colon cancer. It is assumed that the presence of these severity markers is identified especially in patients in advanced stages, to whom preoperative medical or surgical complications may be associated. Of interest is also the research of the hypothesis according to which certain locations of the neoplasia are more frequently associated with perineural or lymphovascular tumor invasions. The presence of the described markers will also be evaluated in tumor stages that allow radical and not palliative intervention, such as the situation of patients in stage IV or with major surgical complications requiring emergency intervention.

4. General research methodology

This material represents a retrospective, observational, non-randomized study, conducted over a period of 5 years, between 2015-2019 inclusive, within the General Surgery Clinic of Colțea Clinical Hospital in Bucharest. The research involved the analysis of a sample of 241 patients with colon cancer at different stages of the disease who underwent curative or palliative surgery, using a classic or minimally invasive approach.

The cases included in this material are representative of a population of patients diagnosed and treated in a tertiary center.

The inclusion criteria in the study were:

- Patients older than 18 years of age,
- Patients with histopathologically typed malignant tumor prior to surgery
- Patients with primary malignant tumor located in the ceco-ascending colon, transverse colon, descending colon, and sigmoid colon.

The exclusion criteria from the study were:

- Patients with malignant tumors in the rectosigmoid,
- Patients with tumoral findings of extracolonic origin but with invasion by contiguity or secondary determinations in the colon that require surgical intervention,
- Patients with incomplete data.

Difficulties encountered in obtaining results:

- Non-compliance of patients at follow-up,
- Lack of interdisciplinary collaboration,
- Identification of a limited number of cases of colon cancer or difficult follow-up throughout the study

5. Study I-Lymphovacular Invasion (LVI)

1. Of the total cases included in the study, 116 male patients and 125 female patients were detected, representing a percentage of 48.13% and 51.86%, respectively. The distribution of cases in the study group was relatively similar, but the total number of cohorted cases is relatively small compared to the data present in the specialty literature, data that indicate a higher incidence of colorectal neoplasm in men compared to women (Choi Y, 2024) (White A, 2018).

2. The age of the patients analyzed ranged from 34 to 92 years. The mean age was 69.14 years, with a median age of 70 years. Rogers JE and Johnson B published a study in 2021 that highlights a decrease in the median age, from 72 years in the early 2000s, to 66 years, a value on a downward slope, as the incidence of colorectal cancer continues to increase in young subjects (Rogers JE, 2021).

3. The most common degree of tumor differentiation in the studied case group was represented by moderately differentiated adenocarcinoma G2 found in 48.9% of cases, followed by well-differentiated adenocarcinoma G1, represented by 44.3% of cases. In current medical practice, moderately differentiated adenocarcinoma is found with the highest frequency, followed by well-differentiated adenocarcinoma (Fleming M, 2012).

4. We identified 24 cases (9.95%) out of the total 241, represented by 11 female cases (45.83%) and 13 male cases (54.16%) with lymphovascular invasion present (LVI+) on the histopathological reports. Specialized studies report the presence of lymphovascular invasion between 5.2 and 89.5%, the percentage variation being in close correlation with the tumor staging of the neoplastic case (Zarbaliyev E, 2024).

5. The most frequent location of LVI+ cases was in the right colon where most cases were detected, namely 13, representing a percentage of 54.1% of the total. The next location was in the sigmoid colon with 8 cases identified by LVI+ representing 33.3%. In 2024 Petrov Krasimir published an article in which he classified the location of lymphovascular invasion according to

the distribution of cases in the right colon, left colon and rectum and identified the ceco-ascending portion of the right colon as the main location of LVI cases (Petrov Krasimir, 2024).

6. Based on the analysis performed, we determined a number of 13 LVI+ cases, representing a percentage of 54.1% of the total patients included in the study with lymphovascular invasion identified with moderately differentiated adenocarcinoma G2. In second place, lymphovascular invasion was identified in tumors represented by well-differentiated adenocarcinoma G1 with a total of 6 cases, representing 25% of the total patients studied.

7. Of the total of 13 cases identified with LVI+ in the right colon, 7 of them were represented by moderately differentiated adenocarcinoma G2, representing a percentage of 53.8%. In the sigmoid colon, out of a total of 8 cases identified with LVI+, 5 cases were detected with the same degree of differentiation, representing a percentage of 62.5%.

8. Lymphovascular invasion was most frequently identified in patients with stage III B neoplastic disease, in 25% of all LVI positive cases.

9. Complications associated with the underlying disease, identified at the time of hospitalization, such as anemia, stenosis and bowel obstruction, presented an OR of lymphovascular invasion almost 3 times higher (Emil-Marian Popescu, 2025a).

10. Patients with colon cancer staged T3 had a 6-fold higher OR of lymphovascular invasion (marginally insignificant), and patients staged T4 had a 24-fold higher OR of vascular invasion. The incidence of cases with lymphovascular invasion increases proportionally with tumor stage T (Wang X, 2021) (Akagi Y, 2013) (Aktekin A, 2015).

11. Patients with N1 staged colon cancer had an OR of 3.18 times higher for lymphovascular invasion, compared to patients with N2 staged cancer who had an OR of 9.5 times higher. Emile SH identified in his study a much higher frequency of lymphovascular invasion in patients with secondary lymph node findings compared to patients detected at N0 stage (Emile SH, 2025).

12. Patients with distant secondary findings had a 5.5-fold higher OR for lymphovascular invasion compared to patients without detected secondary findings. Lymphovascular invasion is

associated with lymph node dissemination and metastatic disease, according to existing data in the literature (Chen K, 2021).

13. The OR for lymphovascular invasion was 2.73 times higher in stage III of the disease (marginally insignificant) and 8.60 times higher for stage IV compared to stages I and II.

14. For every 1 additional lymph node with tumor invasion excised, there is a 17% increase in the OR for lymphovascular invasion. Several studies have reported a poorer prognosis in patients in whom surgery involved the removal of fewer than 12 lymph nodes as recommended by the AJCC in the 8th edition of the TNM staging system (Bozkurt O, 2019).

15. We identified only two independent predictors for the occurrence of lymphovascular invasion, these are represented by tumor stages T and N, information superimposable with the data found in specialized literature, where T stage is considered the most important predictor (Bianchi, 2021).

16. The number of positive lymph nodes detected, excised and evaluated histopathologically, was on average 4 times higher in patients with present lymphovascular invasion compared to patients without invasion.

6. Study II-Perineural Invasion (PNI)

1. Of the total cases included in the study, 116 male patients and 125 female patients were detected, representing a percentage of 48.13% and 51.86%, respectively. The distribution of cases in the study group was relatively similar, but the total number of cohorted cases is relatively small compared to the data present in the specialty literature, data that indicate a higher incidence of colorectal neoplasm in men compared to women (Choi Y, 2024) (White A, 2018).

2. The age of the patients analyzed ranged from 34 to 92 years. The mean age was 69.14 years, with a median age of 70 years. Rogers JE and Johnson B published a study in 2021 that highlights a decrease in the median age, from 72 years in the early 2000s, to 66 years, a value on a downward slope, as the incidence of colorectal cancer continues to increase in young subjects (Rogers JE, 2021).

3. The most common degree of tumor differentiation in the studied group of cases was represented by moderately differentiated adenocarcinoma G2 found in 48.9% of cases, followed by well-differentiated adenocarcinoma G1, represented by 44.3% of cases. In current medical practice, moderately differentiated adenocarcinoma is found with the highest frequency followed by well-differentiated adenocarcinoma (Fleming M, 2012).

4. We identified 34 cases, represented by 15 female cases (44.1%) and 19 male cases (55.8%) with perineural invasion present (PNI+) on histopathological reports. Vakili published a study in 2014 in which the prevalence of perineural invasion in his case series is higher in males compared to females (Vakili M, 2014).

5. The most frequent location of PNI+ cases was in the right colon where most cases were detected, namely 18, representing a percentage of 52.9% of the total. The next location was in the sigmoid colon with 12 cases identified by PNI+ representing 35.2%. Catherine Liebig in her work published in 2009, identifies the most frequent location of cases with perineural invasion present in patients with colorectal cancer, in the rectum, and the next location in frequency in the right colon (Liebig C, 2009).

6. Based on the analysis performed, we determined a number of 18 PNI+ cases, representing a percentage of 52.9% of the total patients studied who were identified with well-differentiated adenocarcinoma G1. Perineural invasion was identified in tumors represented by moderately differentiated adenocarcinoma G2 with a total of 13 cases, representing 38.2% of the total patients. Zozaya reports in his study the relationship between perineural invasion and TNM staging, the degree of tumor differentiation and lymphovascular invasion (Zozaya, 2023).

7. Of the total of 18 PNI+ cases in the right colon, 8 cases were represented by well-differentiated adenocarcinoma G1 representing a percentage of 44.4% and 7 cases of moderately differentiated adenocarcinoma G2 representing a percentage of 38.8%.

8. Perineural invasion was most frequently identified in patients with stage IIIB of neoplastic disease, in a percentage of 26.4% of all LVI+ cases. Perineural invasion is a negative prognostic factor, independently cited in the literature (Chu, 2024).

9. Bowel obstruction, a complication associated with the underlying disease, diagnosed at the time of admission, presented an OR 3.5 times higher for perineural invasion compared to patients in whom it was not identified. In the paper published in 2019, Nozawa describes a close link between intestinal obstruction and perineural invasion and attributes early postoperative relapse to this connection (Nozawa H, 2019) (Emil-Marian Popescu, 2025b).

10. Patients with colon cancer staged T3 had a 4.5-fold higher OR of perineural invasion, and patients with stage T4 disease had a 17.4-fold higher OR. Specialized studies have identified a close statistical association between perineural invasion detected on histopathological examination and the tumor stage T of the tumor (Zahir, 2024).

11. Patients with N1 staged colon cancer had a 2.71-fold higher OR of perineural invasion compared to N2 staged patients who had a 3.90-fold higher OR. A study published in 2024 in the Fortune Journal of Health Sciences, highlights a statistical association with $p<0.001$ for perineural invasion and N1 tumor stage (Mohammad Zillur Rahman, 2024). Perineural invasion is a negative prognostic factor, independent of secondary lymph node findings (Betge J, 2011).

12. Patients with secondary distant findings had a 3-fold higher OR for perineural invasion compared to patients without secondary findings. At the time of diagnosis, according to the literature, patients with positive perineural invasion on histopathological reports have a 5-fold higher risk for secondary distant findings compared to patients without perineural invasion present (Liebig C, 2009) (Emil-Marian Popescu, 2025c).

13. The OR for perineural invasion was 2.08 times higher in stage III of the disease (marginally insignificant) and 4.10 times higher for stage IV compared to stages I and II.

14. For every 1 additional invaded lymph node excised, there is a 3% increase in the OR for perineural invasion (marginally insignificant effect).

15. We identified 3 independent predictors for perineural invasion, these are represented by the presence of bowel obstruction at the time of diagnosis and tumor stages T and N. A 2010 study conducted on a group of 381 patients with colorectal cancer outlines the association between tumor stage T, tumor stage N and perineural invasion (Poeschl EM, 2010).

16. The number of positive lymph nodes detected, excised and evaluated histopathologically was on average approximately 2 times higher in patients with perineural invasion compared to patients without perineural invasion.

7. Conclusions and personal contributions

7.1. Conclusions

According to the statistical analysis of the obtained data, the following were found:

- Patients with lymphovascular invasion present were diagnosed more frequently in the T4 tumor stage of the disease, a similar situation in the case of perineural invasion,
- Both lymphovascular invasion and perineural invasion were detected more frequently in the N1 and N2 disease stages, compared to the N0 stage,
- In the case of lymphovascular and/or perineural invasion, patients were more frequently identified with secondary determinations distant from the primary tumor (stage M1),
- Lymphovascular invasion and/or perineural invasion were identified most frequently in stages III and IV of the TNM classification.

In conclusion, LVI and PNI are mainly associated with advanced colorectal neoplasms. Their detection on postoperative specimens can guide the treating physician regarding the prognosis of the oncological patient and can help in subsequent therapeutic management.

7.2. Personal contributions

1. The most common grade of tumor differentiation encountered in colorectal neoplasm cases is represented by moderately differentiated adenocarcinoma G2 (chapter 5.3, paragraph 2, page 32),

2. The most common location of LVI+ and PNI + cases is in the right colon, followed by location in the sigmoid colon (chapter 5.3, table 5.3, page 34 and chapter 6.3, table 6.3, page 68),

3. The grade of differentiation G2 is most frequently associated with lymphovascular invasion (chapter 5.3, table 5.4, page 35),

4. The grade of differentiation G1 is most frequently associated with perineural invasion (chapter 6.3, table 6.4, page 69),

5. LVI and PNI are most frequently associated with tumor stage III B (chapter 5.3, table 5.6, page 36 and chapter 6.3, table 6.6, page 70),

6. Complications associated with the underlying disease at admission such as anemia, stenosis and bowel obstruction present 3-fold higher OR for LVI (chapter 5.3, paragraph 1, page 38),

7. Patients with bowel obstruction present 3.5-fold higher OR for PNI (chapter 6.3, paragraph 1, page 73),

8. We identified 2 independent predictors for LVI: tumor stage T and tumor stage N (increasing tumor stage T and/or tumor stage N leads to increased OR for LVI) (chapter 5.3, paragraph 1, page 49),

9. We identified 3 independent predictors for PNI: bowel obstruction, tumor stage T and tumor stage N (the presence of bowel obstruction, increased tumor stage T and/or tumor stage N leads to increased OR for PNI) (chapter 6.3, paragraph 1, page 86),

10. For each increase of 1 excised and histopathologically evaluated lymph node, the OR for LVI increases by 17% and for PNI increases by 3% (chapter 5.3, paragraph 1, page 44 and chapter 6.3, paragraph 1, page 79),

11. Patients with secondary distant findings have 5.5-fold higher OR for LVI and 3-fold higher for PNI (chapter 5.3, paragraph 1, page 41 and chapter 6.3, paragraph 1, page 77),

12. The number of tumor-invaded lymph nodes is on average 4-fold higher in patients with LVI and on average 2-fold higher in patients with PNI (chapter 5.3, paragraph 1, page 58 and chapter 6.3, paragraph 1, page 96).

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