Factors which can predicting medical care and survival of patients with colorectal cancer: the role of medical waiting times and new biomarkers

Doctoral (PhD) Thesis

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1. Introduction

1.1 Reasons for the choice of research topic

Colorectal cancer is a major public health problem worldwide. It is the second most commonly diagnosed cancer in men and the third most commonly diagnosed cancer in women worldwide, with 1.2 million new cases per year worldwide (4). Central European countries, including our country, have a high incidence and unfavourable mortality data. In Hungary, an upward trend in incidence has been observed over the last two decades, while mortality has been stagnating with a slight fluctuation (5). Hungary ranks first among European countries in terms of incidence and second in terms of age-standardised mortality. The mortality rate in Hungary is 4-10/10.000 persons per year (EUROSTAT). The median age at diagnosis in Hungary is 70, but this figure is projected to rise in the future. The disease rarely occurs at a young age, but when it does, it is overwhelmingly familial. Only 6.7% of cases occur below the age of 50, but incidence almost doubles every 5 years after the age of 50, with a strong male predominance. Hungary is the leading European country in terms of colorectal cancer incidence and mortality. The gravity of the situation in Hungary becomes even more evident when considering that a significant proportion of deaths from colorectal cancer could be prevented, as the biological features of colorectal cancer development provide the opportunity for early detection of malignant lesions. The development of colorectal tumours is a long, time-consuming process, progressing along the adenoma-carcinoma axis. This means that a polyp that initially shows some degree of cellularity first develops into an in situ lesion that progresses to invasive carcinoma (2) (3). The process takes many years, which, with appropriate screening programmes and effective public information programmes, can give a chance of early diagnosis. Unfortunately, in our country, the majority of colorectal cancers are typically detected at a late stage. It has got reasons that can be connected to the patients and also to the health care system. Therefore, one of the objectives of our study was to identify factors which can be connected to the medical system and analyse the correlations between the symptoms of patients who consult a doctor and the characteristics of their examination and the time intervals to treatment. This will help identify factors that may play a role in the earlier diagnosis of colorectal cancer. Another aim of our study was to identify biomarkers that may be associated with time to metastasis and overall 10-year survival in patients with colorectal cancer.

1.2 Location and symptoms of colorectal tumours

The large intestine is situated in the abdominal cavity like a frame, covered with a peritoneal surface throughout and along its course, extending from the ileocoecal border to the sigma-rectum border. The sigma-rectum border is defined as 16 cm from the anus opening. Colorectal tumours are anatomically classified into 3 groups. Group 1 includes tumours of the right colon: coecum, ascending colon, flexura hepatica and transverse colon. Group 2 includes tumours of the left colon: flexura lienalis, descending colon, sigmoid colon. Group 3 includes tumours of the rectum. The rectal tumours can be further subdivided into lower, middle and upper third tumours according to their location. Their boundaries are, in order, 4-8 cm, 8-12 cm and 12-16 cm from the anus opening. Anal canal processes up to 4 cm are classified as anus carcinomas. 25-45% of colorectal tumours occur in the rectum. The right colon is supplied by the superior mesenteric artery, the left colon by the inferior mesenteric artery. The blood supply to the colon is characterised by a segmental structure consisting of collateral connecting anastomoses. The rectum is supplied with blood by three arteries. The upper one is the inferior branch of the mesenteric artery, the middle one is the internal iliac artery and the lower one arises from the internal pudendal artery. The venous drainage of the colon follows the arteries and leads towards the vena portae. Lymphatic drainage continues through the lymph nodes near the serosa into the retroperitoneal lymph nodes along the aorta. The venous drainage of the rectum is in three directions, the inferior vena cava, the mesenteric vein and the venous plexus surrounding the spine. This explains why primary metastases can form not only inside but also outside the abdominal cavity by haematogenous pathways. Lymphatic drainage is from the upper and middle thirds through the mesorectum by lymphatic drainage along the arteries. From the lower third, especially near the anus, a significant part of the lymphatic drainage is towards the inguinal lymphatic region. Knowledge of these structures is extremely important because radical surgical treatment of colorectal tumours is only safe if these structures are removed along with the tumour, thus reducing the risk of recurrence (1) (6).

Common symptoms of colorectal tumours include abdominal/rectal pain, bloody stools, changes in stool habitus and diarrhoea. There are also less common symptoms such as weakness/fatigue, weight loss and abdominal discomfort. These may be accompanied by abdominal fullness, constipation, nausea or vomiting. Sometimes, obstruction caused by colorectal cancer or ileus may be the first cause of complaint. Colorectal tumours may be asymptomatic for a long time, or metastases may be the first cause of complaints and symptoms, such as bone pain, headache or various neurological symptoms (7, 8).

1.3 Medical care waiting times for colorectal cancer patients

The development of colorectal carcinoma takes a long time: a premalignant lesion can take 10-15 years to develop into a malignant disease. This interval allows for early detection of the disease, for example by screening, and subsequent successful treatment (10). The stage of colorectal cancer at diagnosis is a major predictor of patient survival (11, 12). A 5-year survival rate of 80-90% has been observed for colorectal carcinomas detected at an early stage (stages I-II). In advanced (stage III) forms, this figure is 50-60%. At metastatic (stage IV) stage, 5-year survival is only 10-12% (13).

Delays in the correct diagnosis of colorectal carcinoma are partly due to patient-related delays and partly due to delays in healthcare. Patients may remain asymptomatic for months (14) or their symptoms may be trivialised, ultimately leading to patient-related delays (15-18). Patients most commonly present to their doctor with bowel-specific symptoms, such as rectal bleeding, abdominal pain or changes in stool habitus. Some patients present to the health system with general symptoms such as weight loss, loss of appetite or fatigue (19, 20).

Other reasons for delay in diagnosis of colorectal carcinoma may be linked to healthcare. According to a study by Lyratzopoulos and colleagues, one third of patients with colorectal carcinoma present three or more times to their GP before being referred to a specialist, compared to only 17.9% of all cancers (21). Inadequate diagnostic resources, including a lack of imaging and trained endoscopists, further increase waiting times for healthcare.

Waiting times from symptom onset to diagnosis and treatment of cancer have been studied in several Western countries (22-26). Some countries have formulated and implemented guidelines on waiting times for diagnosis and treatment. In Denmark, for example, it is recommended that a maximum of 14 days should elapse between referral and colorectal histological diagnosis and that treatment should start within a further 14 days at the latest (27). In the UK, guidelines state that treatment of cancer patients should start within 2 months of referral by a GP (28).

Screening of the asymptomatic population for colorectal carcinoma has been shown to reduce colorectal carcinoma mortality and has proven to be a valuable method for preventing colorectal cancer-related deaths, and colorectal carcinoma screening strategies have been developed in many countries (29-31). In our country, pilot model screening programmes have been conducted in the general population since 2000 (3), but controversies surrounding the adoption

of one- or two-step screening procedures have hindered the introduction of a systematic population screening programme (32). Although it is now possible to screen for colorectal cancer in principle throughout the country, in practice, comprehensive screening has not yet been achieved.

In Hungary, patients with colorectal carcinoma are more likely to die from their disease than in any other European country (3, 32, 33). The high morbidity and mortality rates associated with colorectal carcinoma in the Hungarian population justify an investigation of the underlying causes.

1.4 Role of predictive factors in the response to treatment of rectal tumours

The outcome of locally advanced rectal cancer is related to the response to neoadjuvant radiochemotherapy, but this response varies from patient to patient (34). Because of individual differences, it is therefore essential that patients who are not expected to respond to neoadjuvant radiochemotherapy do not undergo unnecessary treatment, as this will only delay time to surgery and will not result in adequate tumour regression. Predicting the response to treatment and using personalised therapy would be optimal in the treatment of these patients, in order to avoid wasting time and avoid side effects of neoadjuvant radiotherapy, and therefore several studies in recent years have aimed at predicting the expected response to neoadjuvant treatment, identifying so-called predictive markers (35).

Different studies evaluate diseases and the results of therapies along different time intervals, with overall survival being defined as the total life expectancy from diagnosis. The 5 or 10-year overall survival indicates the percentage of patients who are alive in the 5th or 10th year after diagnosis, regardless of whether or not the patient has an active tumour at the time of diagnosis. It has been shown that the prognosis of colorectal carcinoma and the expected survival of the patient are closely related to the local extent of the tumour and the stage of the tumour (36). Some clinical parameters and molecular markers have been identified as predictors of overall survival in previous studies (36-41). For example, the lymphocyte/monocyte ratio (LMR) has been associated with survival in different cancer types, including colorectal cancer, according to several studies (42-45).

Growth Hormone Releasing Hormone (GHRH) is a peptide hormone produced by the hypothalamus and is present in various tissues and tumours. Tumour tissue contains significantly more GHRH receptors than normal tissue. GHRH stimulates the secretion of

growth hormone (GH) by binding to GHRH receptors in the anterior pituitary (46-48). GH stimulates the production of insulin-like growth factor I (IGF-I), which plays a major role in malignant cell transformation and metastasis during the development of various cancers (49, 50).

GHRH receptor and splice variants have been shown to be present in several cancer cell lines (46, 48). GHRH is considered an autocrine/paracrine growth factor for tumours, as its presence has been demonstrated in breast, endometrial, ovarian, colon, gastric and lung cancers (51). Therefore, targeted interference against the GHRH/GHRH-R signalling pathway may have antitumour effects in some cancers (52). The antitumor effects of GHRH inhibitors have been investigated in vitro in cell lines under experimental conditions (53). For example, GHRH inhibitors inhibit GH secretion and autocrine GHRH binding to GHRH receptors on tumor cells, thereby reducing tumor-secreted IGF-I production (54-56).

Heat shock proteins (Hsp) are members of a large family of proteins found in all living things. Cells produce heat shock proteins in response to different types of stresses that they are under. Examples of such stresses include environmental changes, extreme temperatures, anoxia, hypoxia, and various chemical agents that can induce protein denaturation in cells (57). In such cases, heat shock proteins act as molecular chaperones, promoting the stabilization of cellular proteins and thus playing an important role in cell protection and survival, i.e., they have antiapoptotic effects (58). Hsps are ATP-dependent proteins with ATPase activity, except for small heat shock proteins (sHsps) (59). Heat shock proteins can be grouped according to their molecular weight. Small heat shock proteins (sHsps) have low molecular mass (between 2 and 43 kD). Like the other Hsps, they are molecular chaperones and play a role in the development of several types of tumours (60, 61).

Hsp90 is also a molecular chaperone that ensures the stable conformation of several client proteins that may play a role in the signalling pathways responsible for malignant cell progression (62). The client proteins include EGFR, IGF-1R, CDK4, Akt, ERbB2, c-Met, BCR-ABL, RET, Fms-like tyrosine kinase 3 (FLT3), BRAF, NF-kB, Raf-1, HER2/Neu, NPM-ALK, neuronal nitric oxide synthase (nNOS). Thus, Hsp90 has become a much studied molecule in cancer research (58, 59, 63, 64). Increased expression of Hsp90 has been demonstrated in several tumour types, including oropharyngeal squamous cell carcinoma, multiple myeloma, lung, breast, ovarian and pancreatic cancer (58, 59). Research suggests that increased

expression of Hsp90 may serve as a biomarker for esophageal, lung and bladder cancers with poor prognosis (65).

First described by our group a few years ago, sHsp16.2 was shown to be expressed in neuroectodermal tumors (40). In a later study, a clear correlation was found between its increased expression and the grade of brain tumors, indicating that it may be a potential biomarker for brain tumors (39, 40). Hsp16.2 also has a direct mitochondrial stability enhancing effect and can potentiate its action by binding to Hsp90 (41).

Another potential target for anti-cancer therapy is the Akt signalling pathway, as it is often over-expressed in tumour cells. The Akt signalling pathway is considered to be one of the most important pathways inhibiting apoptosis (66, 67).

The SOUL protein, also first described by our group, is a member of a BH3 domain protein family and promotes necrotic cell death by altering mitochondrial permeability under oxidative stress (68). BH3 domain Bcl-2 proteins are thought to play a role in mitochondrial apoptosis. Bid and Bim bind to Bax-like proteins on the outer mitochondrial membrane via their BH3 domain. This induces conformational changes in the proteins and affects the integrity of the inner membrane without oligomerization and permeabilization of the outer mitochondrial membrane. Interactions between the lipid bilayer and members of the proapoptotic Bcl-2 family contribute to the apoptotic process. While Bcl-2 family members only affect apoptotic folymats, the Bax protein is able to influence necrotic and apoptotic cell death by itself. SOUL, a BH3-domain protein, induces cell death and necrotic cell death in response to certain stimuli, such as oxidative stress (68, 69).

The identification of new protein molecular markers may be important in rectal cancer because they may be potential biomarkers predicting the expected tumour response to neoadjuvant radiotherapy and because there may be a correlation between protein expression levels and the overall survival of the patient. In a previous study, our research team analysed the expression levels of GHRH-R, Hsp 90, p-Akt, Hsp16.2 and SOUL in pre-treatment rectal tumour samples. The results suggested that GHRH-R and Hsp90 were independent predictive factors of histopathological response to neoadjuvant radiochemotherapy in patients with locally advanced rectal cancer (36).

2. Objectives

2.1 Aims of the research

"Characteristics of access to treatment for colorectal cancer patients in a pilot study in Baranya County"

Taking into account the high morbidity and mortality rates of colorectal cancer in Hungary, the aim of our study was to analyse the association between the first symptoms and characteristics of colorectal cancer patients and the time interval to therapy in a pilot study.

"Expression of tumour-associated proteins predicting survival in patients with locally advanced rectal cancer"

Another aim of our study was to identify proteins that are potential biomarkers of disease outcome, essential for personalising and increasing the efficacy of treatment of locally advanced colorectal cancer. We investigated the relationship between the intensity of protein expression (GHRH-R, Hsp90, Hsp16.2, p-Akt and SOUL) in pre-treatment tumour samples and the 10-year overall survival (OS) and time to metastasis. We also aimed to investigate whether patients' time to metastasis and 10-year overall survival (OS) were associated with certain clinical parameters (gender, time to surgery, tumour location).

2.2 Our research questions

"Characteristics of access to treatment for colorectal cancer patients in a pilot study in Baranya County"

1. What was the relationship between the specialty of the doctor who started the colorectal cancer investigation and the nature of the symptoms?

2. What was the relationship between the stage of colorectal cancer and the frequency of symptoms?

3. What was the relationship between the stage of colorectal cancer and the specialty of the investigating physician?

4. How did the median value of the Interval to Therapy vary according to the stage of colorectal cancer and the specialty of the investigating physician?

"Expression of tumour-associated proteins predicting survival in patients with locally advanced rectal cancer"

1. Is there a correlation between GHRH-R, Hsp 90, Hsp 16.2, SOUL and p-Akt expression and histopathological response to neoadjuvant radiotherapy in locally advanced rectal tumours?

2. Is there a correlation between certain clinical parameters and histopathological response to neoadjuvant radiochemotherapy in locally advanced rectal cancer?

3. Is there a correlation between GHRH-R, Hsp 90, Hsp 16.2, SOUL and p-Akt expression and 10-year survival in locally advanced rectal cancer?

4. Is there a correlation between some clinical parameters (histopathological response, tumour localisation, gender) and 10-year survival in locally advanced rectal tumours?

5. Can any clinical parameter or protein expression be identified as an independent prognostic marker of survival?

6. Is there a correlation between certain clinical parameters (histopathological response, tumour localisation, sex), the expression of the proteins tested and the time to metastasis in locally advanced rectal cancer?

3. Methods

3.1 "Characteristics of access to treatment for colorectal cancer patients in a pilot study in Baranya County"

Ethics authorisation

The retrospective study was performed with the permission of the Regional Scientific and Research Ethics Committee of the University of Pécs Clinical Centre (ethical permission number: 2017/6744).

Test plan

In our study, retrospective data collection was performed on colorectal cancer patients of general practitioners in Baranya County within a 5-year interval (2012.01.01.-2016.12.31). All general practitioners in Baranya County received two notifications about the possibility to participate in the study, and 26 general practitioners volunteered to collect data on colorectal cancer patients in their practices. The participating GP practices received three documents before the data collection started: a leaflet describing the objectives of the study, a Microsoft Excel spreadsheet with the data to be collected and a consent form, which was returned to the study manager after completion.

Data collection

The data were collected mainly from the database of general practitioners' practices, and additional information was collected from the eMedSol database of the University of Pécs Clinical Centre (PTE KK). Data from patients with colorectal cancer of any stage (according to the International Classification of Diseases) within the given time interval and with a diagnosis of "C" code within the given interval or within the preceding 5 years maximum were included. A total of 390 colorectal cancer patients' data were collected. Patients under the age of 18 years, or those who had a previous colorectal cancer recurrence within the time interval, or whose data were missing from the data to be analysed for some reason, were excluded from the study. For this reason, data from 212 patients were finally analysed in the present study. The following data were extracted from GP practices and from the eMedSol database of the PTE KK: (1) demographic data, (2) patient's symptoms at the time of first (investigating) doctor's visit (3) speciality of first (investigating) doctor (4) stage of tumour at diagnosis (5) date of initiation of

anticancer therapy. The first doctor was considered to be the doctor who first met the patient and initiated the investigation (e.g. laboratory, abdominal ultrasound, etc.) and/or the first step of treatment, who could be the general practitioner, emergency physician or any other specialist. Tumour staging was based on histology, imaging studies and the official Oncoteam opinion, according to the 8th European Society for Medical Oncology Lower gastrointestinal cancers guidelines. The starting date was the first doctor-patient encounter, when the patient first presented to the doctor with symptoms suggestive of colorectal cancer. If the patient was free of complaints, the first doctor-patient encounter was considered to be the first GP visit when the patient first received a referral from the doctor (e.g. laboratory, abdominal ultrasound, etc.) that triggered the investigation of the patient for colorectal cancer, and the diagnosis of cancer was then confirmed as a result of the investigations. The Time to Intervention (TEI) is the time (number of days) from the starting date to the initiation of therapy. The initiation of therapy was defined as the date on which any form of anticancer therapy (surgical, oncological) was started. Data were recorded and then checked by two independent researchers to ensure the accuracy of the data.

Data analysis, statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows 24. Descriptive analysis (frequency and cross tabulation analysis) and analysis of variance were performed. Statistical significance was calculated using Kruskal-Wallis and Fisher tests. The results of the tests were considered statistically significant at $p<0.05$.

3.2 "Expression of tumour-associated proteins predicting survival in patients with locally advanced rectal cancer"

Ethics authorisation

The study was performed with the permission of the Regional Scientific and Research Ethics Committee of the University of Pécs Clinical Centre (ethics permission number: 7532-PTE 2019).

Methods, patients, tumour samples

The study was conducted at the Clinical Centre of the University of Pécs, which is the dedicated oncology centre (including surgical and oncotherapy departments) for the South Transdanubian region. A total of 114 patients with locally advanced (cT3/T4 and/or cN+ and cM0) rectal cancer

were included in our study (69 patients between January 2005 and December 2006 and 45 patients between January 2009 and March 2010). All patients received neoadjuvant radiochemotherapy (NRCT) followed by surgery. Pre-treatment examinations included digital rectal examination, rectosigmoidoscopy or colonoscopy, biopsy, abdominopelvic CT, pelvic MRI, chest X-ray or CT. 3D planned conformal radiotherapy was performed in all cases with a "belly board" positioning device in the prone position, using 18 MV photon irradiation. 3D conformal irradiation of the primary tumour and regional lymph nodes was performed for 5 weeks in 25 fractions at a radiotherapy dose of 45/1.8 Gy. As part of the radio-chemotherapy, patients received 500 mg/m2 5-fluorouracil by continuous infusion and 30 mg/m2 folic acid bolus on days 1-5 of weeks 1 and 5 of radiotherapy. Four weeks after completion of neoadjuvant radiochemotherapy, patients were restaged and surgical resection was performed 6-9 weeks after completion of neoadjuvant therapy in a total of 109 patients (5 patients were excluded from the study). Curative resection was performed in all cases. All patients completed a consent form, which was approved by the local ethics committee. The main clinical characteristics of the patients who underwent surgery are shown in the Table.

Esetszám	**N =109**
Kor (év)	
≤ 60	60 (55%)
> 60	49 (45%)
Nem	
férfi	55 (50%)
nő	54 (50%)
Klinikai T stádium	
cT2	4 (4%)
cT3	93 (85%)
cT4	12 (11%)
Klinikai N stádium	
cN0	41 (38%)
cN1-2	68 (62%)
Távolság a végbélnyílástól (cm)	
<5	34 (31%)
5-10	43 (40%)
>10	32 (29%)
Műtétig eltelt idő (hét)	
≤ 7	64 (59%)
>7	45 (41%)

Histopathological evaluation

For histological evaluation of resected specimens, the response system developed by Mandard et al (70) was used to determine the pathological response to neoadjuvant treatment. The five-point tumour regression grading (TRG) is based on the presence of residual tumour cells and the degree of fibrosis. The TRG classification includes: TRG1 (complete regression) is defined as the absence of residual tumour and fibrosis in different layers of the rectal wall, TRG2 is characterised by the rare presence of residual tumour cells scattered throughout the fibrosis, TRG3 shows a higher number of residual tumour cells already present but fibrosis still predominates, TRG4 shows residual tumour overgrowth of fibrosis, TRG5 shows complete absence of tumour regression. In line with previous studies, to facilitate statistical analysis, TRG response was divided into two groups: TRG1-2: good tumour response, while TRG 3-5: poor tumour response (70-72).

Production of polyclonal antibodies against Hsp16.2 and SOUL

Rabbits were subcutaneously immunized with 100 pg of recombinant Hsp16.2/GST and SOUL/GST fusion protein prepared by the working group at the Institute of Medicinal Chemistry and Biochemistry, PUE UAS, as previously described (39, 69, 73). Booster injections were given at 4-week intervals with 50 pg of protein per injection. After blood collection and centrifugation, antisera were stored at -20°C. IgGs were purified by protein G-Sepharose chromatography (Sigma) according to the manufacturer's protocol.

Immunohistochemistry

Sections of pre-treatment tumour tissue samples were fixed in formalin and embedded in paraffin. They were then incubated with the following primary antibodies. The GHRH-R antibody detected the presence of both GHRH-R and GHRH-R splice variants. The p-Akt and total Hsp90 polyclonal rabbit primary antibodies were purchased from Cell Signaling and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and the proprietary anti-Hsp 16.2 and anti-SOUL polyclonal primary antibodies were used. Immunohistochemical staining was performed according to the strepatavidin-biotin-peroxidase method with hydrogen peroxide/ 3-amino-9-ethylcarbazole predevelopment using the Universal kit described previously (74). Only secondary IgG was incubated with control sections. Slides were evaluated using an Olympus BX50 light microscope with an integrated imaging system (Olympus Optical Co., Hamburg, Germany). Staining intensity was recorded semi-quantitatively as mild (+), moderate (++) or

strong (+++) staining as previously described (75). Normal cellular and vascular structures of the samples were used as internal positive controls. All slides were evaluated by the same experienced pathologist who was not familiar with the clinical and pathological data of the patient.

Data collection, categorisation

In order to increase the number of patients per group for the analyses, the categories of the different variants were pooled: age over 60 vs. 60 years or younger, cT2 vs. cT3 vs. cT4, cN0 vs. cN1- 2, distance from anus less than 5 cm vs. between 5 and 10 cm vs. more than 10 cm, time to surgery within 7 weeks vs. beyond 7 weeks. For statistical analysis, immunohistochemical intensity values were divided into low intensity (0, +) and high intensity (++, +++) categories. Overall survival (OS) was calculated from the time of diagnosis to the time of death from any cause. Time to metastasis was defined as the time interval from the time of diagnosis to the first metastasis(s).

Data on patient parameters and survival were obtained from the clinic's electronic medical database (eMedsol database) and the National E-health Infrastructure Database (EESZT). In cases where data on survival were not available from the above-mentioned databases, a member of our research team contacted the patient or family members by telephone to obtain information. Contact details were also obtained from electronic health systems.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences 16.0 software (SPSS, Chicago, IL). Chi-square test for the degree of tumor regression and clinical response was used to compare clinical parameters and biological markers. The relationship between clinical and biological markers and overall survival (OS) and time to metastasis were assessed using Kaplan-Meier curves, and the level of significance was determined using a log-rank test. Probability values (p) < 0.05 were considered statistically significant.

4. Results

4.1 "Characteristics of access to treatment for colorectal cancer patients in a pilot study in Baranya County"

Abdominal/rectal pain was the most common symptom reported by patients attending the emergency department, while patients visiting their GPs mentioned bloody stools most often. Patients presenting to the emergency department were diagnosed with a significantly higher proportion (61%) of late-stage (stage III-IV) tumours than patients presenting to their GP (57.3%). The TEI was shorter when patients presented to an emergency department (median TEI: 15 days for late (stage III-IV), 34.5 days for early (stage I-II) cancer) than when they first presented to a GP (median TEI: 86 days for late, 83 days for early stage cancer).

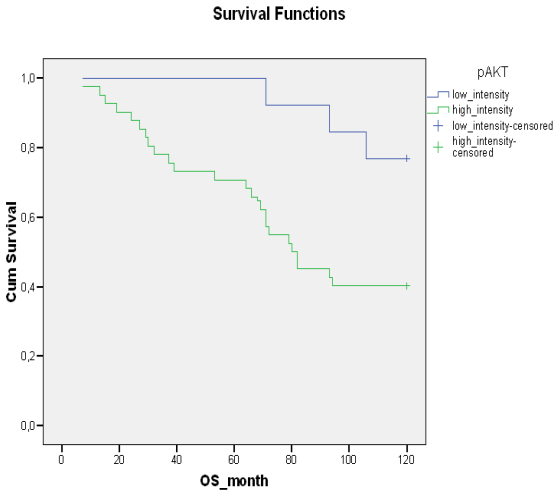
Tünetek	**n (%) Esetszám** **Háziorvos indította (127)**	**n (%) Esetszám** **Sürgősségi szakorvos indította (49)**	**Fisher teszt** **(p)**
Hasi/végbéltáji fájdalom	31 (24,4)	18 (36,7)	*0,133*
Véres széklet	41 (32,3)	8 (16,3)	*0,039*
Gyengeség/fáradtság	27 (21,3)	9 (18,4)	*0,835*
Fogyás	29 (22,8)	3 (6,1)	*0,009*
Hasmenés	28 (22,0)	7 (14,3)	*0,197*
Széklethabitus változás	18 (14,2)	2 (4,1)	*0,066*
Hasi teltségérzet	9 (7,1)	7 (14,3)	*0,150*
Székrekedés	8 (6,3)	2 (4,1)	*0,728*

	Daganat stádiuma	Esetek száma (%)	TEI átlag (nap)	Szórás	TEI medián (nap)
Háziorvos	I-II	47 (42,7)	98,19	66,40	83,00
	III-IV	63 (57,3)	100,06	66,37	86,00
Sürgősségi szakorvos	I-II	16 (39,0)	40,81	38,75	34,50
	III-IV	25 (61,0)	23,12	21,30	15,00
Kruskal-Wallis teszt P< 0.001					

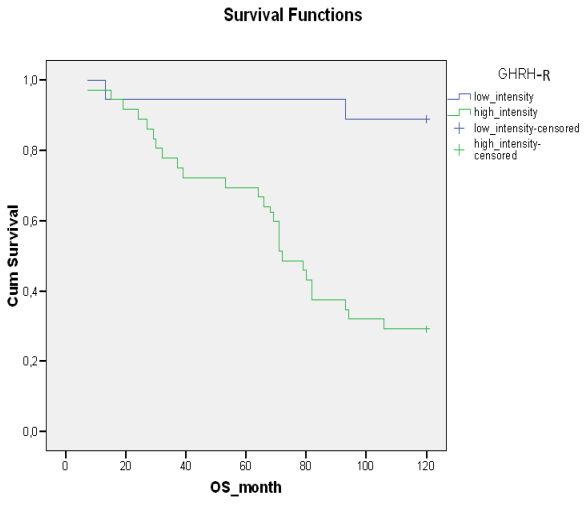
4.2 "Expression of tumour-associated proteins predicting survival in patients with locally advanced rectal cancer"

Increased expression of p-Akt, GHRH-R and Hsp90 was significantly correlated (p=0.001; p=0.000; p=0.004;) with lower 10-year survival (1a, 1b, 1c), and high expression levels of p-Akt and GHRH-R were significantly correlated with shorter time to metastasis (3a, 3b). Tumours localised to the lower third of the rectum were significantly associated with both longer time to metastasis and improved 10-year survival (4b).

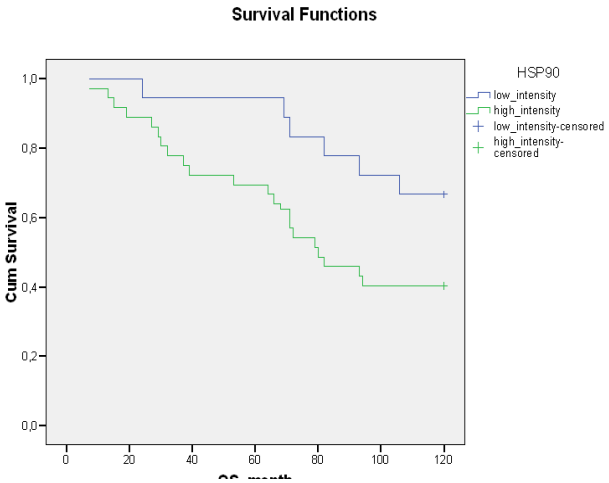
1a



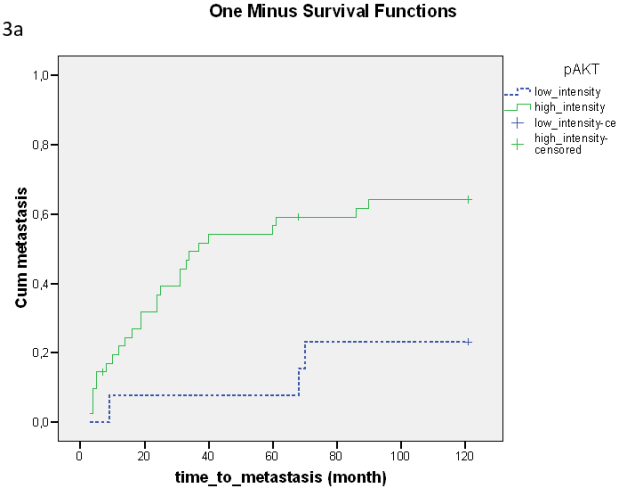
1b



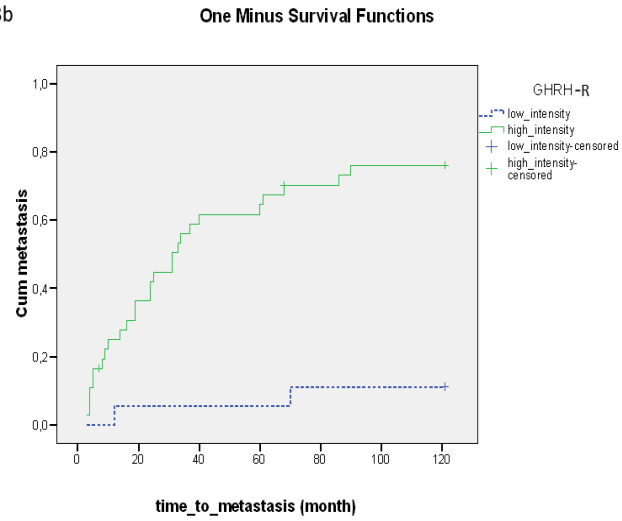
1c


Survival Functions
HSP90
low_intensity
high_intensity
low_intensity-censored
high_intensity-censored
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120

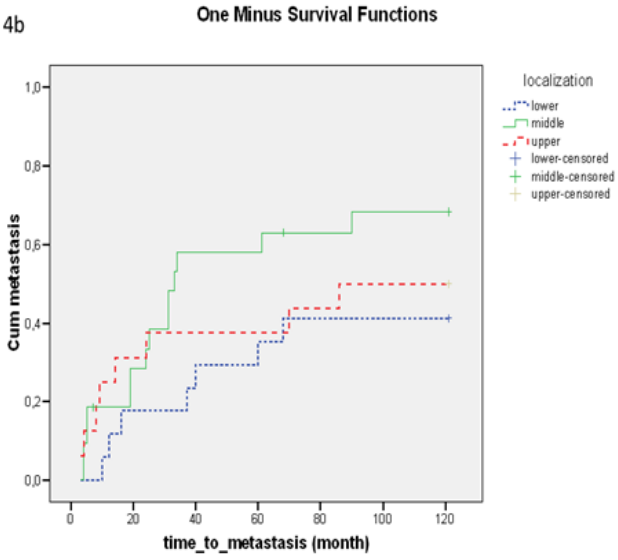
3a


One Minus Survival Functions
pAKT
low_intensity
high_intensity
low_intensity-ce
high_intensity-censored
Cum metastasis
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
time_to_metastasis (month)

3b


One Minus Survival Functions
GHRH-R
low_intensity
high_intensity
low_intensity-censored
high_intensity-censored
Cum metastasis
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
time_to_metastasis (month)

4b


One Minus Survival Functions
localization
lower
middle
upper
lower-censored
middle-censored
upper-censored
Cum metastasis
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
time_to_metastasis (month)

5. Conclusions

To our knowledge, our study is the first to analyse medical care waiting times for colorectal carcinoma in a Central and Eastern European country. Our pilot study both investigated the association between colorectal cancer symptoms and shed light on possible causes of the high morbidity and mortality rates of colorectal cancer.

Abdominal/rectal pain was the most common symptom in patients presenting to the emergency department, which may also be associated with a higher proportion of late-stage tumours (e.g. obstructive tumours). Patients were more likely to present to their GP with symptoms of weight loss and bloody stools than to an emergency physician, and to report more than one symptom to their GP at the same time. Patients with cancer presenting to the emergency department received treatment significantly faster than patients presenting to their GP, which may be due to differences in the characteristics of the two care systems, patient referral and investigation options. Based on our study, the intervals to treatment observed in case of emergency or GP visits in Baranya County were similar to those in Western European countries. In colorectal cancer, the most important prognostic factor at diagnosis is the stage of the tumour [34-36]. We concluded that delay in seeking medical attention is a major factor behind the high mortality rates in our country.

Our results highlight the importance of prevention. The development of colorectal cancer is a long process, lasting up to 10-15 years, which makes this type of cancer an excellent candidate for screening. Early detection of colorectal cancer symptoms is an essential element in reducing patient-related delays. For this reason, in addition to the introduction of public awareness campaigns to promote cancer prevention, patient education to promote early detection of symptoms is essential. Another form of prevention, the introduction of a regular national colorectal screening programme, could also significantly help to detect colorectal cancer at an early stage.

Our further research was the first to report that Hsp 90, p-Akt and GHRH-R are potential molecular predictive markers of overall survival in patients with locally advanced colorectal cancer. Their increased expression in pre-treatment tumour samples was associated with reduced overall survival. Furthermore, GHRH-R and Hsp90 are independent prognostic factors for 10-year overall survival, and p-Akt may also have a predictive role. Increased GHRH-R and Hsp90 expression were significantly associated with reduced 10-year overall survival.

We have also shown that increased expression of p-Akt and GHRH-R is a prognostic factor for the time to metastasis. These results, together with our previous finding that GHRH-R expression was an independent prognostic factor for response to therapy (12), suggest that GHRH-R may play a particularly important role as a molecular biomarker. An important feature of our study is the particularly long follow-up period of 10 years, which demonstrates the long-term impact of the biomarkers studied on disease outcome. In addition, our study may have implications for further research as it adds to existing data on potential biomarkers for colorectal cancer and may provide a basis for further studies involving larger patient populations. Finally, our findings may also have potential practical and clinical value. The advantageous properties of biomarkers include that they can be easily and non- or minimally invasively obtained from patients, are widely available and low cost. Detecting the expression of biomarker proteins by immunohistochemistry can be a simple and cost-effective way to determine prognosis and potentially even aid in therapy planning, as it is available in medical centres that routinely diagnose and treat cancer patients and no additional patient samples are required, as biopsies are automatically taken for histopathology and diagnosis. GHRH-R and Hsp90 appear to be promising biomarkers for locally advanced rectal cancer. However, further research, larger and validated studies are needed to confirm our results.

6. Referencies

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7. Publications

7.1 Publications related to the thesis

Pozsgai E, Busa C, **Fodor D**, Bellyei S, Csikos A. Wait times to diagnosis and treatment in patients with colorectal cancer in Hungary. Cancer Epidemiol. 2019 Apr;59:244-248. doi: 10.1016/j.canep.2019.02.015. Epub 2019 Mar 5. PMID: 30849616.

IF: 2.89, Q2

Fodor D, Busa C, Cservenák N, Kiss I, Bellyei S, Csikós Á, Pozsgai É. A colorectalis daganatos betegek kezeléshez jutásának jellemzői egy Baranya megyei pilotvizsgálat keretében [Characteristics related to the treatment of colorectal cancer patients based on a pilot study in Baranya county, Hungary]. Orv Hetil. 2021 Jan 24;162(4):153-160. Hungarian. doi: 10.1556/650.2021.31982. PMID: 33486467.

IF: 0.707, Q4

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Number of first authored works related to the thesis impact factor: 5.464

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IF: 0.4, Q3

Total impact factor of all publications: 8.754

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