UNIVERSITY OF MEDICINE AND PHARMACY
CRAIOVA

THESIS FOR DOCTORAL DEGREE (PhD)

The contribution of endoscopic ultrasonography and immunohistochemical factors in the diagnosis, staging and prognosis rectal cancer

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- THESIS RESUME -

-2012-
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Keywords: Rectal cancer, endorectal ultrasonography, staging accuracy, immunohistochemistry, Her2/neu VEGF, Ki-67, p53, survival time.

GENERAL PART

INTRODUCTION

Diagnosis and early detection of CRC is one of the major goals of medical activity and should become a priority for health systems because in the prognosis of CRC are involved many factors such as the histological type of cancer, size, location, degree of invasion, loco-regional metastasis and metastasis in other organs.

Chapter I

EPIDEMIOLOGY OF COLORECTAL CANCER

In epidemiological terms and evolutivity, colorectal cancer (CRC) is one of the major public health problems in more countries. Currently, colorectal cancer is the most common digestive tract cancer (13% of all malignancies) and the second cause of cancer death in the U.S. and Western European countries. In these countries the risk of developing colorectal cancer during life is about 5%.

In the United States 142 570 people were diagnosed in 2010 with colorectal cancer and about 39 670 patients were diagnosed with rectal cancer. Estimates for 2011 show 101,340 new cases of colon cancer and 39,870 new cases of rectal cancer.

Chapter II

ETHIOPATHOGENY OF COLORECTAL CANCER

Colorectal cancer develops as a result of genetic and epigenetic changes occurring within 10-15 years. About 75% of patients with colorectal cancer are sporadic cases without presenting evidence that would inherit the disease. The remaining 25% of patients have family history of colorectal cancer, which suggests either the contribution of genetic factors or common exposure to environmental factors or a combination of both factors.
Chapter III

MORPHOPATHOLOGY OF COLORECTAL CANCER

Rectal adenocarcinoma is the most common histopathological type of rectal cancer with 98% of cases. Other types are represented by lymphomas (1.3%), gastrointestinal stromal tumors (0.3%) and carcinoid tumors (0.4%). Rectal neoplasms expand locally by progressive penetration of the bowel wall. Expanding of the primary tumor is intramural and parallel to the long axis of bowel.

Chapter IV

CLINICAL MANIFESTATIONS

Rectal tumors have a slow growth rate and can be a long time until they grow enough to cause symptoms. Symptoms which appears in rectal cancer can be summarized as follows:

- impregnation, neoplastic, non-specific symptoms;
- characteristic symptoms of rectal tumors;
- symptoms due to complications.

Chapter V

DIAGNOSTIC OF COLORECTAL CANCER

Diagnosis of rectal cancer is based on digital rectal examination, simple or double contrast barium enema, sigmoidoscopy or colonoscopy and histopathological results. Computed tomography (CT), endorectal echoendoscopy (ERUS) and magnetic resonance imaging (MRI), positron emission tomography (PET) are techniques used in preoperative staging of rectal cancer.

SPECIAL SECTION

CLINICAL AND IMAGISTIC STUDY

64 consecutive patients who had biopsy-proven rectal carcinoma were included in our group of study. For 51 patients (32 males and 19 females) we could compare the ERUS classification with histopathology results. Their mean age was 62.8±11.31 years (ranging between 37 to 87 years).
Regarding the localization of the primary tumor, in 18 patients (35.3 %) the tumor was found in the upper third, in 12 patients (23.5%) in the middle third and in 21 patients (41.2 %) in the lower third of the rectum.

At ERUS examination 23.6% of the patients were staged as uT2, 68.6% were staged as uT3 and 7.8% of the tumors were classified as uT4 (4 patients). None of the patients were staged as uT1. The histological study of the resection specimens revealed pT1 tumors in 4 patients (7.8 %), pT2 in 17 patients (33.3 %), pT3 in 28 patients (54.9 %) and 2 patients had pT4 tumors (4%). The comparison between ERUS and histopathological findings is illustrated in Table I. The overall accuracy of ERUS for T staging was 66.7 % (k=0.545, p=0.01) with 29.4% of tumors being overstaged (15 patients) and 3.9% downstaged (2 patients). The highest accuracy was found for T2 (58.3%) and T3 tumors (71.4%). Comparing the different tumor sites in relation to the distance from the anal verge the accuracy of ERUS for staging tumors in the upper third was 66.67 %, in the middle rectum 83.3% and in the lower third 38.09 %.

ERUS examination identified positive criteria for lymph node involvement in 47.06% of the patients (23 patients with 1 to 3 positive nodes and one patient with 4 invaded nodes). Histopathological analysis classified 18 patients (35.3%) as having pN1 stage disease and 5 patients (9.8 %) as pN2 (Table II).

Table I ERUS versus histopathologic staging of rectal wall involvement

<table>
<thead>
<tr>
<th></th>
<th>pT1 (n)</th>
<th>pT2 (n)</th>
<th>pT3 (n)</th>
<th>pT4 (n)</th>
<th>Total (n)</th>
<th>Overstaged (%)</th>
<th>Understaged (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>uT1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>uT2</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>12</td>
<td>25</td>
<td>16.6</td>
<td>58.3</td>
</tr>
<tr>
<td>uT3</td>
<td>1</td>
<td>9</td>
<td>25</td>
<td>0</td>
<td>35</td>
<td>28.6</td>
<td>0</td>
<td>71.4</td>
</tr>
<tr>
<td>uT3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>17</td>
<td>28</td>
<td>2</td>
<td>51</td>
<td>29.4</td>
<td>3.9</td>
<td>66.6</td>
</tr>
</tbody>
</table>

The accuracy for N staging was 74.51%, with a sensitivity of 73.91% and a specificity of 75%. The negative predictive value was 77.77% and the positive predictive value was 70.83% (Table II). Kappa value for N staging was 0.504 corresponding to a moderate correlation with a standard error of 0.075.
Table II. ERUS versus histopathology for staging lymph nodes

<table>
<thead>
<tr>
<th></th>
<th>pN positive</th>
<th>pN negative</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>uN positive</td>
<td>17 (33.3%)</td>
<td>7 (13.72%)</td>
<td>24 (47.05%)</td>
<td>Positive predictive value 70.83 %</td>
</tr>
<tr>
<td>uN negative</td>
<td>6 (11.7%)</td>
<td>21 (41.17%)</td>
<td>27 (52.94%)</td>
<td>Negative predictive value 77.77 %</td>
</tr>
<tr>
<td>Total</td>
<td>23 (45.09%)</td>
<td>28 (54.90%)</td>
<td>51 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73.91 %</td>
<td>74.51%</td>
</tr>
</tbody>
</table>

IMUNOHISTOCHEMICHAL AND HISTHOLOGICAL STUDY

Tumour tissues from 46 cases of rectal carcinoma (32 male, 14 female patients; mean age 72.5 years) were investigated. All patients had been admitted to the Emergency County Clinical Hospital of Craiova in 2008. The follow up period was 3 years.

The first parameter examined by the histopathological exam was the tumor location. Middle segment of the rectum was the most affected with 48% of cases, followed in order by the upper and lower segment. Survival analysis revealed a bigger mean survival in patients with tumors located in the inferior rectum and lower survival duration to those with tumors located in other segments.

According to WHO’s classification system, more than three quarters of microscopic aspects of the biopsy fragments were adenocarcinoma with tubular pattern. The mucinous patterns were present in 13% of cases followed by papillary in 8.7% of cases. The squamous carcinoma was the rarest, with only 2 cases. The analysis of cancer survival with histopathological types showed a similar median survival rate at patients with tubular adenocarcinoma and mucinous adenocarcinoma.

The vast majority of cases were classified as well differentiated neoplasms with more than 50% of cases, followed by the moderately differentiated neoplasm with almost a third of cases (31%). Analysis of survival correlations showed that differentiated forms(in varying
degrees) had time to death about two years. Well and poorly differentiated forms had similar median survival time (23 months), four months lower than moderately differentiated.

Almost half of cases in our study were staged as T3 tumours, 39% as T2 and 9% as T1 tumours. Analysis of survival correlation with the degree of rectal wall invasion showed a reverse trend of the average survival in relation to the rectal wall invasion depth. Thus, patients with stage T1 tumors had an average survival of about three years, followed in descending order by patients in stage T3 and T2.

8 patients have liver metastasis. As expected, patients who have liver metastasis have the lowest median survival (21 months).

Positive nuclear immunohistochemical staining for Ki-67 antibody was evident in all the 46 carcinomas examined. Ki-67 index of investigated tumors ranged from 15% to 85% with a mean Ki-67 index of 49.78% indicating a significant variation in proliferative activity in colorectal cancer. Graphical analysis of survival by survival curves revealed a clear difference of survival between the group with reduced Ki67 and intense Ki67 staining.

Immunohistochemical expression of p53 was negative in 14 cases (30.43%) and positive in 32 cases (86.36%). 14 cases have been identified with low expression of p53 (15-40% positivity) and 18 cases with intense expression of p53 (more than 50% positivity). Analysis of survival curves showed a higher survival rate for p53-positive patients at one year survival rate, in subsequent periods of follow up was lower than in the negative.

Immunohistochemical expression of VEGF was positive in 28 cases (60.86%) and negative in 18 (39.14%). In terms of gender there was a predilection of positive VEGF expression for males and advanced tumor stages, T2 and T3 respectively.

In our study, positive VEGF expression has been shown to increase with decreasing of the degree of differentiation. Analysis of correlation of VEGF expression with survival showed that patients with tumors who didn’t have expressed VEGF factor had a higher survival than those who had expressed this marker.

Her 2/neu staining intensity was positive in 26 cases (56.52%) and was absent in 20 cases (43.48%). Analysis of the degree of invasion in the wall (tumor stage T) shows an increase in correlation with increasing Her 2/neu staining. Survival curves were also calculated with a clear difference between the groups, the group with negative HER2 expression presented the longest
median survival, followed by those with low and moderate intensity staining for HER2/neu. This tendency was confirmed statistically by Chi-square index = 7.32(p = 0.02).

CONCLUSIONS

Accurate preoperative staging is mandatory in selecting the optimal treatment modality for patients with rectal cancer. The depth of tumor invasion into the rectal wall and the number of involved lymph nodes are critical prognostic factors for patients with rectal cancer and essential parameters to be considered in guiding their treatment. In our study we found a 66.7% accuracy for all T stages, in moderate concordance to the histopathologic findings of the resection specimens. As a common finding with previous studies most misinterpretations resulted from overstaging of the tumors (29.4%). This high rate of overstaging is usually due to the peritumoral inflammatory reaction which cannot be easily differentiated from the tumor itself on ERUS examination. Another possible explanation for the difficulty in correctly classifying these patients is the hypoechoic appearance of the tumor which is not very easy to separate from the hypoechoic layers of the rectal wall, especially the muscularis propria. Understaging of the tumor was found in 3.9% of cases and this is probably explained by the microscopic invasion of the rectal wall which cannot be detected by ERUS.

We found ERUS to be the most accurate for T2 and T3 stages, i.e. locally advanced tumors that do not invade adjacent organs. The lower accuracy in T4 cases could be due to the small number of patients included and the uneven distribution of tumors by stages, this representing a limitation for our study. Regarding tumor location in terms of the distance from the anal verge and its impact on the endosonographic assessment of wall invasion, previously published results are inconclusive. In our study the accuracy of ERUS in assessing the depth of rectal wall invasion was also higher for middle and proximal tumors as compared to the distal ones, with the difference being statistically significant (88.3 % and 66.67 % vs 38.09%, respectively). An explanation for the difference in staging accuracy is that the typical endosonographic five-layer structure of the rectal wall is somehow less well-defined just above the anal canal. Also the anatomy of the ampulla recti makes endosonographic examination more difficult from a technical point of view. One cause of misinterpretation is the presence of reactive inflammatory lymph nodes which are difficult to differentiate from malignant lymph nodes as there is an overlap between their echo features. Also smaller nodes might be missed during ERUS examination while metastatic foci have been reported in approximately 18% of nodes with a diameter of less than 5 mm. We found that ERUS could predict nodal involvement in rectal cancer patients with 74.51% accuracy (PPV 70.83%, NPV 77.77%), despite the cutoff value of 10 mm for lymph node diameter, higher than the one used in other studies. Although in our study ERUS had slightly lower overall staging accuracy than
previously reported, we found that it can predict with high accuracy T2 and T3 tumors. As this is a highly operator dependent technique and there is a learning curve, with wide use in the general practice results are likely to improve. In conclusion ERUS is a useful method for the evaluation of rectal cancer, especially for selecting patients in need for neoadjuvant treatment. With wider use and further technical developments its current limitations can be overcome. Limitations of the current study were the radial EUS scope and the limited number of patients with T1 and T4 lesions.

To improve prognosis in colorectal cancer, basic research and molecular genetics, with colorectal screening and the widespread use of noninvasive techniques, are some new or relatively new directions to be developed.

From the 46 cases studied, 32 cases were still alive at the end of the follow up period. The survival rate at 1 year after diagnosis was 78.3%, it decreased to 69.5% at the end of second and third year of follow up. Statistical analysis of patient’s survival correlation showed that in the studied group there were no significant differences in survival between the sexes. The lowest median survival was recorded in younger patients. The most affected segment of the rectum was the middle third with 48% of cases, followed in order by the upper and lower third.

Positive nuclear immunohistochemical staining for Ki-67 antibody was evident in all 46 resected specimens. Graphical analysis of survival by survival curves revealed a clear difference of survival between the group with low Ki67 staining and intense Ki67 staining. In colorectal cancer, were reported conflicting results between Ki67 expression and prognosis and survival. Palmqvist and colleagues demonstrated that a low proliferation index in Dukes stage B tumors is associated with a poor prognosis compared with tumors with high proliferation index. These tumors showed a good response to radiotherapy, radiation preferentially destroy tumor cells in division. Other studies such as that conducted by Kimura and colleagues in 2000 showed a poor prognosis in colorectal tumors with high proliferative index. Also, high proliferation index is a prognostic factor after surgical resection with curative intent. Immunohistochemical expression of p53 was negative in 14 cases (30.43%) and positive in 32 cases (86.36%). The data are consistent with the literature, mutations of p53 protein were found in 40-60% of rectal cancers. P53 value varied inversely with degree of differentiation. Patients with positive p53 expression showed a median survival rate lower than to the patients with negative p53 expressions.

Immunohistochemical expression of VEGF was positive in 28 cases (60.86%) and negative in 18 cases (39.14%). Positive VEGF expression was associated with advanced tumor stages. Recent studies have shown direct correlation between VEGF expression and aggressiveness of rectal cancer, Takahashi et al. showed that VEGF expression level in patients with colorectal cancer without lymph node invasion is associated with recurrence and Cascinu et al. confirmed that positive VEGF is associated with reduced survival at 5 years.
Her 2/neu staining in the analyzed cases was positive in 26 cases (56.52%) and was absent in 20 cases (43.48%). Analysis of the degree of invasion in the wall (tumor stage T) showed an increase in intensity with increasing intensity of Her 2/neu staining. Survival curves were also calculated with a clear difference between the groups, the group with negative HER2 expression presented the longest median survival, followed by those with low and moderate intensity staining for HER2/neu. HER-2/neu overexpression was associated with tumor size (> 5 cm), degree of differentiation, lymphatic and vascular invasion, with a higher recurrence rate and a poor survival (Demirbas et al). Other studies (Jesus et al) showed that HER-2/neu expression is not correlated with gender, age, degree of tumor differentiation, location of primary tumor and overall survival.
CURRICULUM VITAE

Personal information

First name(s)/ Surname(s)  Pirvu Daniel-Cristian
Adress  St. 1 Decembrie 1918, no. 37, Bl. T5, App. 10, 200173 Craiova(Romania)
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Email  pirvu_daniel2005@yahoo.com
Nationality  Romanian
Date of birth  03.04.1983
Gender  Male

Work experience

Dates  01 October 2008- 30 September 2011
Occupation or Position held  PhD Student
Main activities and responsibilities  Research activities in the field of rectal cancer, endoscopy and echoendoscopy
Name and address of employer  University of Medicine and Pharmacy of Craiova
St. Petru Rares No. 2, 200349 Craiova (Craiova)

Type of business or sector  Education
Dates  15 October 2009- Present
Occupation or
Position held: Teaching assistant Department of Internal Medicine - Gastroenterology

Main activities and responsibilities: Research activities in the field of gastroenterology, teaching activities for

Name and address of employer:
- University of Medicine and Pharmacy of Craiova
  St. Petru Rares No. 2, 200349 Craiova (Craiova)

Type of business or sector: Education

Dates: 01.01.2009 - Present

Occupation or Position held: Resident physician

Main activities and responsibilities: Patient care. Diagnosis and treatment in the field of Internal Medicine, Gastroenterology, Pneumology and Cardiology

Name and address of employer:
- County Emergency Hospital of Craiova, St. Tabaci No. 1, 200642 Craiova (Romania)

Type of business or sector: Medical

**Education and training**

Dates: 01 October 2008 – 30 September 2011

Title of qualification Awarded: PhD Degree

Principal subjects/ Occupational skills Covered: Devising and applying novel endoscopic techniques in the staging of rectal
Devising medical-oriented database systems for patients informations.
Interpretation of contrast enhanced ultrasonography.

Name and type of organization providing education and training
University of Medicine and Pharmacy of Craiova
St. Petru Rares No. 2, 200349 Craiova (Craiova)

Dates
01 October 2002- 15 September 2008

Titles of qualification awarded
Doctor Medic

Principal subjects/occupational skills covered
Training in fundamental research techniques
Training in diagnostic techniques and methods
Training in patient care and treatment modalities for general medicine
Gained extensive theoretical and practical knowledge in the field of general medicine

Dates
15 September 1998- 17 July 2002

Titles of qualification awarded
Bachelor Degree

Principal subjects/occupational skills covered
General subjects and basic general occupational skills

Name and type of organization providing education and training
Charles the 1st National College(College)
St. Ioan Maiorescu no. 2, 200418 Craiova(Romania).

Personal skills and competences

Mother tongue(s)
Romanian

Other languages

Self assessment

<table>
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<tr>
<th>Language</th>
<th>Understanding</th>
<th>Speaking</th>
<th>Writing</th>
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<tr>
<td>English</td>
<td>Proficient user</td>
<td>Proficient user</td>
<td>Proficient user</td>
</tr>
<tr>
<td>Deutsch</td>
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</table>
Social skills and competences
Team spirit gained over 12 years of working in various settings, during professional exchanges and internships.
Good ability to adapt to multicultural environments, gained through six years as voluntary in an internships.
Good ability to adapt to multicultural environments.
Good communication skills gained through multiple local, national and trans-national collaborations and meetings.
Good social adaptability through internships.

Technical skills and competences
Experience in operating various types of Olympus endoscopes and echoendoscopes gained through the training stage in Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Magdeburg, Germany.
Experience in immunohistochemistry and immunofluorescence techniques and laboratory apparatus.
Experience in interpreting videocapsule endoscopy results, two years involved as collaborator in the national research grant “Comparision between video capsule endoscopy and push-and-pull enteroscopy in patients with small bowel pathology. Experience in general clinical patient management, gained through my medical residency.

Computer skills and competences
Good command of image and sequence analysis, with special focus on histology and medical imaging (Analysis Pro and Nikon suites).
Advanced programming skills (C++, Turbo Pascal).
Advanced knowledge of medical statistical programs (IBM SPSS Statistics, MedCalc).
Good command of advanced editorial and image manipulation (Microsoft Office, Adobe Creative Suite, Corel applications etc).
Good knowledge of web design and site creation and administration.

Artistic skills and competences
Amateur photographer

Other skills and competences
Chess
Football (professional competitive play during college).

Articles published in journals:

  
  [http://www.rjme.ro/RJME/jsp/currentiss.jsp#a](http://www.rjme.ro/RJME/jsp/currentiss.jsp#a)
• Vere CC, Cazacu S, Streba CT, Sima F, Pîrvu D, Ionescu A. Capsule Endoscopy: Diagnostic Role in Obscure Gastrointestinal Bleeding Current Health Sciences Journal. 2009, Nr. 3 (Revistă cat. B, indexată CNCSIS)

