

**University of Medicine and Pharmacy of Craiova  
Doctoral School**



**DOCTORAL THESIS**  
**The prognostic implication of VEGFR1 and  
VEGFR2 in pancreatic cancer**

**ABSTRACT**

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**CRAIOVA  
2015**

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## References

## **I. STATE OF KNOWLEDGE**

### **Chapter 1. Introduction. Risk factors. Pathogenesis**

#### **Introduction**

Pancreatic cancer is one of the most important public health problems worldwide, being the fourth leading cause of cancer death, with 1-year and 5-year survival rates of 25% and 5%, respectively. EUS is the most effective method used in the management of pancreatic cancer. Angiogenesis plays a crucial role in tumor development and VEGF and its receptors (VEGFR1 and VEGFR2) are the most important angiogenesis stimulating factors in pancreatic cancer [1 – 4]. The purpose of this study is to improve knowledge concerning the role of endoscopic ultrasonography in the evaluation of pancreatic adenocarcinoma, to determine VEGFR1 and VEGFR2 gene expression and to identify prognostic factors involved in pancreatic cancer.

**Keywords:** pancreatic cancer, endoscopic ultrasonography, fine needle aspiration, angiogenesis, VEGFR1, VEGFR2.

#### **Etiology. Risk factors. Pathogenesis**

There are many risk factors that are involved in the development of pancreatic neoplasms: smoking, alcohol, diet (high total energy intake, red meats, intake of total fat as well saturated fatty acids and monounsaturated fatty acids) and obesity, genetic factors (familial pancreatic cancer, hereditary pancreatitis, BRCA2 mutations etc), pre-existing benign diseases (diabetes mellitus, chronic pancreatitis etc) [5 – 17].

More than 95% of malignant neoplasms of the pancreas develops from exocrine elements of the gland (ductal and acinar cells) and they are called adenocarcinomas. Pancreatic tumors are found predominantly in the pancreatic head (60-70%) having an average size of 25 – 35 mm. Whatever the location, the tumor develops insidiously and produce symptoms late. The natural history of the disease leads to loco-regional invasion of neighboring anatomical structures and finally to metastasis (liver, peritoneal cavity, etc.) [18 – 23].

## **Angiogenesis**

Angiogenesis is a key process in tumor development. There are many factors that stimulate tumor neovascularization process, but the most important is VEGF (vascular endothelial growth factor). VEGF protein family includes currently several members who are involved in the development of most digestive or nondigestive cancers. VEGF-A, VEGF-C, VEGF-D and VEGF receptors VEGFR1 and VEGFR2 are particularly involved in pancreatic cancer. VEGFR-2 is the most important receptor in angiogenesis assessing in pancreatic cancer, being a negative prognostic factor [24 – 36].

## **Chapter 2. Diagnosis**

### **Clinical manifestations**

Pancreatic cancer develops insidiously and becomes symptomatic, late in the evolution, with compression / invasion of anatomical locoregional structures. The main clinical manifestations are represented by obstructive jaundice and abdominal pain. It may also occur: weight loss, exocrine pancreatic insufficiency (steatorrhea and malabsorption) and endocrine (diabetes) and less commonly, depression, migratory thrombophlebitis (Trousseau sign), venous thrombosis and gastrointestinal bleeding [37 – 40].

### **Imaging diagnosis**

Imaging currently occupy the central and decisive role in the management of pancreatic cancer being involved in all stages: detection and characterization of pancreatic masses, identify any anatomical variants, evaluation of locoregional extension, detection of metastasis and evaluation of tumor resectability. The main modalities of imaging for the detection of pancreatic cancer are abdominal ultrasound (US), endoscopic ultrasonography (EUS), endoscopic retrograde cholangiopancreatography (ERCP), computer tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET-CT) [41 – 48].

### **Endoscopic ultrasonography**

Described in the early 1980s (by DiMagno), EUS has become the main technique for evaluating pancreatobiliary disorders and has proved to have a diagnostic yield higher than US, CT, MRI and PET-CT for recognising early pancreatic tumors. Pancreatic adenocarcinomas typically have the EUS appearance of a *heterogeneous, hypoechoic mass with irregular margins*. EUS represents *the most sensitive and specific of the imaging*

***procedures currently available*** in the management of pancreatic masses (accuracy, sensitivity and VPN up to 98%).

EUS has the ability to detect small lesions (2 – 5 mm in diameter). Furthermore, color flow imaging in Doppler or power Doppler mode can be used to describe the peripancreatic vasculature. Thus, ***pancreatic adenocarcinomas are typical hypovascular***. The major advantage of Doppler examination is obvious during fine needle aspiration, helping to avoid puncture of an important blood vessel and thus of consecutive bleeding complications. Use of ***contrast enhancement in EUS (CE-EUS)***, based on second-generation microbubble ultrasound contrast agents (SonoVue), has been shown to improve the characterization of the vasculature inside the lesion of interest, to better delineate benign from malignant pathology. CE-EUS has two major vascular phases: arterial phase (parenchymal / pancreas) and venous phase. Pancreatic ductal adenocarcinoma appears hypovascular in both phases, whereas pseudotumoral chronic pancreatitis or neuroendocrine tumors are either isovascular or hypervascular as compared with the surrounding parenchyma. ***Real-time sonoelastography*** allows appreciating tissues hardness during EUS, with high accuracy in the differential diagnosis of solid pancreatic tumors. Thus, pancreatic cancer has, typical, hard consistency. EUS elastography was also reported to be useful for the differentiation of focal pancreatic masses, especially in pseudotumoral chronic pancreatitis and pancreatic cancer, especially in the presence of false negative EUS-guided FNA results and a strong suspicion of pancreatic cancer [49 – 62]. The major advantage of EUS is offered the opportunity to obtain cytological / histological material by EUS-FNA.

### **Chapter 3. Fine needle aspiration (EUS - FNA)**

Fine needle aspiration (EUS – FNA) performed under EUS guidance, is a minimally invasive technique that allows the collection of cytological / histological from pancreatic masses [50]. Described for the first time in 1991, FNA is done routinely today in most centers of gastroenterology, proving that this procedure has a decisive impact in the management of pancreatic cancer. Although the negative predictive value is relatively low (46 – 80%), EUS – FNA accuracy for the diagnosis of pancreatic ductal adenocarcinoma ranges from 80 - 95%, with a sensitivity and a specificity of 85% and 98%, respectively.

In addition to the advantage of providing a diagnostic cytological / histological, EUS - FNA provides enough material to determine gene expression (by qRT – PCR) of molecular markers of angiogenesis (ex. VEGFR1 and VEGFR2) [63 – 79].

## **Chapter 4. The prognosis of pancreatic cancer**

Although there have been remarkable technological progress in improving the diagnosis of pancreatic cancer over the last decades, the survival rate at 5 years, remains only 5%. The modest prognosis is caused mainly by the advanced stage at the diagnosis, more than 80% of patients presenting with locally advanced or metastatic disease, which precludes surgery. The prognosis remains reserved even at the operated patients (15 – 20% of cases). Thus, the need to discover new diagnostic and therapeutic methods is essential [80 – 83].

## **II. PERSONAL CONTRIBUTIONS**

### **Chapter 5. Objectives. Material and method**

#### **Objectives.**

The research theme chosen is of great importance, given the devastating prognosis of pancreatic cancer and the need to identify more effective diagnostic and therapeutic methods is crucial. The purpose of this study is to improve knowledge concerning the role of endoscopic ultrasonography in the evaluation of pancreatic adenocarcinoma, to determine VEGFR1 and VEGFR2 gene expression and to identify prognostic factors involved in pancreatic cancer.

#### **Material and method**

This was a prospective study of 108 consecutive patients, with clinical and imaging suspicion of pancreatic neoplasms. The study was conducted over a 4 years period (January 2011 - December 2014) in the Research Center of Gastroenterology and Hepatology Craiova, Romania. The patients are individualized into 3 groups: group I (pancreatic cancer, n = 68), control groups (group II - chronic pancreatitis, n = 20 and group III - normal EUS pancreas, n = 20). All the patients, who fulfilled the inclusion criteria for the study, were examined by endoscopic ultrasonography (in standard mode, Doppler, by contrast and elastography). After the tumor characterization, stadialization and determination of tumor resection status, EUS guided FNA was performed for cytology and some of the samples were stored in the genetics lab, where later it was determined the VEGFR1 and VEGFR2 gene expression. The patients were carefully followed up and by consulting the national database regarding the population mortality, they were determined the survival rates.

The results of the examinations were structured in a **Microsoft Excel** database (software package Microsoft Office 2010 Professional) to be later statistically processed,

using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and **IBM Statistic Package for the Social Sciences (SPSS)** version 20.0 (IBM Corporation, Armonk, NY, USA).

Initially we performed descriptive statistics: the frequency, the lowest value, the highest value, the mean, the median, standard deviation, coefficient of variation, standard error of mean, 95% confidence interval.

Subsequently, we used univariate analysis, contingency table analysis with a chi-square test, where we calculated the statistical significance p (considering p value <0.05 statistically significant) and in the end we conducted a Cox regression model. Cox regression estimates the effect of a statistical parameter on a particular event (event considered final or end-point). Because the analysis of the evolution of patients for this study, there are multiple correlations between the explanatory variables introduced in the Cox model, we used Backward analysis method, by which, in several steps, it eliminates the variables that do not contribute significantly to model, remaining, finally, only those variables that best explain the relationship with the final event analyzed (inoperable status, 6 months survival, 12 months survival and mortality of the patients with pancreatic cancer).

## **Chapter 6. Results.**

### **Demographic characteristics**

Patients with pancreatic cancer had a mean age of 62.51 years at the time of diagnosis. Most patients with pancreatic cancer are men, from urban areas and aged between 60 – 79 years.

### **EUS characteristics**

Pancreatic tumors diagnosed have an average size of 34 mm in diameter and they had the cephalic location in most cases. Endoscopic ultrasound is the best method for locoregional staging in pancreatic cancer. Thus, the majority (67%) of the patients were in III and IV stages at the diagnosis time, only about 1 of 5 patients, meeting the criteria for operability.

EUS demonstrated a diagnostic accuracy of 93% (using Doppler mode, elastography and CE-EUS). EUS – FNA has an accuracy of 81% and an excellent specificity (100%) in the diagnosis of pancreatic adenocarcinoma. However, FNA has a modest negative predictive value, 22% of the pancreatic cancers having a false negative cytology. In this group of patients, EUS has an important role in guiding the pancreatic cancer diagnosis.

## **Genetic results**

Based on real-time qPCR analysis, VEGFR1 and VEGFR2 expressions were present in 90% and 65% of the analysed samples obtained through EUS-guided FNA, respectively.

## **Survival in pancreatic cancer**

Almost 90% of patients died during the study, with a median survival rate of only 9 months. The survival was directly influenced by the initial stage and by the presence of VEGFR1 and VEGFR2 gene expression. Thus, the patients with positive VEGFR1 expression have an average survival of 7.22 months compared to those with negative VEGFR1 (17.25 months). The situation was similar for positive VEGFR2 (5.96 months) versus negative VEGFR2 (12.42 months)

## **Prognostic factors in pancreatic cancer**

The non-resectability was individually correlated statistically with: hypoechogenic appearance at conventionally EUS examination with the presence of VEGFR1 expression with the mortality and with survival less than 12 months after diagnosis. There is also a weak association with male patients, tumor cephalic location and the survival under 6 months. Using input parameters, sex of patients, conventionally EUS appearance, tumor location and VEGFR1 expression, Cox regression model analysis validates *the presence of VEGFR1 gene expression as predictive factor for non-resectability*.

The survival of less than 6 months from the moment of diagnosis is individually associated with the positive result of the FNA and with the tumor invasion. There is also a weak correlation with the presence of lymph node invasion (N1) and with the inoperability status. The Cox model demonstrated that *the VEGFR1/VEGFR2 co-expression is a predictive factors for survival less than 6 months from diagnosis*.

It is observed that *the presence of genetic expression of VEGFR1, VEGFR2 and coexpression VEGFR1 / VEGFR2 are predictors of survival of less than 12 months from the moment of diagnosis*.

The Cox model shows that the *co-expression of VEGFR1 / VEGFR2 is the factor with the greatest prognostic implications in pancreatic cancer mortality*.

## **Chapter 7. Discussion.**

The present study confirms the literature data, and demonstrated that VEGFR1 play an important role in tumor progression [24, 83, 84], being a predictor of inoperability status and of survival of less than 12 months from the moment of diagnosis. The published studies reported that VEGFR-2 is the most important receptor in the assessment of angiogenesis in pancreatic cancer. VEGFR-2 expression in tumor cells is correlated with tumor invasion into surrounding tissues and with the negative prognosis [85]. This paper confirms that VEGFR2 is a predictive factor of survival of less than 12 months from the moment of diagnosis. ***Moreover, co-expression of VEGFR1 / VEGFR2, is the the most important negative prognostic factor in terms of the survival of less than 6 months from the moment of diagnosis and of the mortality in pancreatic cancer.***

Pancreatic cancer is therefore one of the deadliest cancers.

***EUS examination remains the gold standard for management of pancreatic cancer, realizing the diagnosis, the staging and determination of resectability with the highest accuracy*** [86]. Technological advances are far from being stalled. The discoveries from the last time evolved to the possibility to obtain real time optical biopsy of the solid pancreatic masses during EUS (confocal laser endomicroscopie) [87]. The progresses in terms of the survival rate are slow, but they exist. The present study reports a survival rate at 1 year, slightly higher (29%) compared to published literature results (25%). Therefore, further studies for the discovery of new diagnostic and therapeutic alternative are vital. Given the implication of VEGF and its receptors in pancreatic cancer angiogenesis, antiangiogenic therapy must be the cornerstone of treatment in this type of cancer. In conclusion, the fight with this pathology is not completely lost and therefore should not be abandoned. In the UK approximately 4,300 patients with pancreatic cancer were still living at the end of 2006 after 10 years from diagnosis [4]. For this reason, hopes to prolong survival in these patients still exist, and the efforts should be focused, in equal measure, on primary prevention (prevention of modifiable risk factors such as smoking and harmful diet), on early diagnosis, on accurate preoperative staging (development of diagnostic techniques - EUS) and improving therapeutic options (the discovery of new anti-angiogenic therapies, developing of the nano-ablation etc.).

## **Final conclusions:**

- ❖ This study confirms the poor prognosis of pancreatic adenocarcinoma, almost 90% of patients dying during the study, with a median survival rate of only 9 months
- ❖ EUS is the most effective technique in the diagnosis and staging of pancreatic cancer with an accuracy that can reach up to 93%. Moreover, EUS has an important role in guiding the pancreatic cancer diagnosis at the patients with clinical suspicion of cancer and false negative FNA.
- ❖ Based on real-time qPCR analysis, VEGFR1 and VEGFR2 expressions were present in 90% and 65% of the analysed samples obtained through EUS-guided FNA, respectively.
- ❖ The survival was directly influenced by the initial stage and by the presence of VEGFR1 and VEGFR2 gene expression.
- ❖ VEGFR1 was found to be a negative predictive factor for tumor resectability and for the survival of less than 6 months from diagnosis.
- ❖ VEGFR2 was identified as a predictor for survival of less than 12 months from diagnosis.
- ❖ The VEGFR1 / VEGFR2 coexpression was the factor with the largest negative prognostic implications for the survival at 6 months and for the mortality in pancreatic adenocarcinoma.

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