**UNIVERSITY OF MEDICINE AND PHARMACY**

**„CAROL DAVILA" BUCHAREST**

**DOCTORAL SCHOOL**

**MEDICINE**

# EARLY DIAGNOSIS OF PANCREATIC CANCER
# PHD THESIS SUMMARY

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# Introduction

Pancreatic cancer is one of the most aggressive malignancies, with 5-year survival rates that do not exceed 10% [1,2]. The poor prognosis is primarily attributable to the late diagnosis, in stages beyond the therapeutic stage, as well as a poor response to oncological treatments [1,2]. On the other hand, incidence and prevalence rates for pancreatic cancer are low. Under these circumstances, the implementation of screening strategies is not feasible from a cost-effectiveness perspective [3,4]. However, the American Society of Gastroenterology and Endoscopy's most recent guideline from 2022 recommends screening for pancreatic cancer in high-risk patients, namely those with genetic susceptibility [5]. In this subpopulation, annual evaluation by echoendoscopy or nuclear magnetic resonance is recommended, with the age of implementation of the screening strategy varying between 35 - 50 years depending on the underlying condition [5]. The proportion of patients with genetic susceptibility to pancreatic cancer, compared to the total number of patients diagnosed with this neoplasia, is small, at around 10% [5].

Recent research has centered on the identification of potential non-invasive biomarkers with a role in the early diagnosis and prognostic evaluation of these patients [6,7]. The primary objective of these studies is to increase the proportion of patients diagnosed at a stage amenable to surgical treatment, which is presently the only curative therapeutic method. Thus, the diagnostic and prognostic potential of certain proteomic markers, free circulating DNA, and microRNA has been established [6,7].

One of the conditions with a risk of developing pancreatic cancer is chronic pancreatitis [8]. Also, the similarity between clinical manifestations, imaging, and biochemical changes can lead to a delay in the diagnosis of pancreatic cancer, initially wrongly categorized as chronic pancreatitis [8].

Considering all these aspects, we aimed to identify some non-invasive biomarkers with a role in the early diagnosis of pancreatic cancer and, secondarily, improve the prognosis of these patients. Thus, we developed the PhD thesis titled "**Early diagnosis of pancreatic cancer**", which is divided into two sections: a general section that offers data on the current state of knowledge in this subject and a special section that presents personal contributions.

The general part is subdivided into two chapters that present epidemiological and etiopathogenic data, respectively, on the diagnostic management of pancreatic cancer. These data were published as review articles in ISI journals with an impact factor [7,9].

*Chapter 1* presents the incidence, prevalence, and mortality rates of pancreatic cancer worldwide, the histopathological classification of pancreatic cancer, the principal risk factors for this malignancy, and the pathophysiological mechanisms that support their participation in pancreatic oncogenesis.

*Chapter 2* offers data on the clinical and imaging diagnosis of pancreatic cancer, but also the importance of molecular diagnosis in the evaluation of patients with this malignancy. The challenges facing research in this field are also highlighted, namely the low incidence of pancreatic adenocarcinoma and the need for large national and international collaborations to obtain a substantial number of biological samples, necessary for the identification of new biomarkers [10]. A meta-analysis published in 2021 highlights the heterogeneity of pancreatic tumors and the need to develop biomarker panels to increase their sensitivity, specificity, and diagnostic accuracy [10].

The section related to individual contributions is organized into five major chapters. The first two chapters present the working hypothesis, the general objectives, and the gener al methodology of the research, while the final three chapters present the results of the clini cal and experimental studies conducted during the doctoral research. At the end of the PhD thesis, conclusions and personal contributions are highlighted, and bibliographic references and appendices are presented in a separate section.

## Chapter 3: Working hypotheses and general research objectives

Pancreatic cancer is one of the most aggressive forms of cancer, with very high death rates. The negative prognosis is mainly due to the late diagnosis in advanced stages, beyond the therapeutic stage, but also to the poor response to oncological treatments. On the other hand, implementing pancreatic cancer surveillance strategies is not cost-effective.

Using biological samples from pancreatic adenocarcinoma patients already diagnosed with the disease can lead to the identification of biomarkers that indicate the presence of symptomatic disease [10]. An alternative strategy is to use biological samples from patients with premalignant lesions (pancreatic intraepithelial neoplasia, intraductal papillary mucinous neoplasm, or mucinous cystic neoplasm) or pancreatic diseases with proven malignant potential (chronic pancreatitis) [10].

The study of the molecular changes that occur during the sequential progression from the initial lesion to pancreatic cancer can improve our understanding of the pancreatic oncogenesis process [10]. It can also provide a basis for the design of future research strategies, with the objective of identifying biomarkers with a role in the early diagnosis of

this malignancy [10]. Moreover, the use of risk groups as controls and the combination of molecular markers with clinical characteristics may lead to the identification of biomarkers capable of selecting populations at increased risk of developing pancreatic cancer [10]. The final objective is the extension of patient subgroups in which it is feasible to implement screening strategies for the early detection of pancreatic cancer. The most commonly used biological sample for the identification of new biomarkers is blood, due to its accessibility and low cost [11].

Thus, there is an imperative need to identify groups in the general population that are at increased risk for pancreatic cancer in order to implement strategies for their careful follow-up [12]. A series of clinical studies have reported better survival rates in patients with subcentimeter pancreatic tumors compared to patients with advanced disease [13]. Additionally, treatment options for patients with resectable cancer continue to expand, including the availability of multimodal neoadjuvant therapy as well as the development of adjuvant chemotherapy regimens [13]. Under these conditions, increasing the percentage of patients diagnosed at an early stage, from 15-20% at present, to 50% could lead to a substantial improvement in their survival rates [13].

Taking into account all of the above, we sought to identify potential non-invasive biomarkers that could be extensively used to improve the prognosis of patients with pancreatic cancer. In light of the correlation between chronic pancreatitis and the risk of progression to pancreatic cancer, as well as the paucity of comparative studies between the two patient groups, we decided to investigate biomarkers with potential in the advanced selection of patients at risk of malignant transformation. Thus, we established three research cohorts: patients with chronic pancreatitis, patients with pancreatic cancer, and healthy subjects with similar age and gender distributions to the patients in the first two groups. Our study also considered the cost-effectiveness ratio, which was a crucial factor. Under these conditions, we focused on the use of blood samples that were easy to collect, posed no significant risks for the patient, and involved low costs.

*The main aim* of the study is to identify potential non-invasive, cost-effective biomarkers with a role in the early detection of pancreatic cancer. Consequently, we analyzed the expression of panels of plasma proteins, cytokines, chemokines, growth factors, angiogenesis markers, and other soluble proteins, as well as the expression of micro-RNAs that target the most relevant signaling pathways and genes associated with oncological conditions.

Among the secondary objectives, we mention:

a. Identifying risk factors for malignant transformation in chronic pancreatitis patients.
b. A comparison of the plasma profiles of cytokines, chemokines, growth factors, and other soluble proteins in three groups of patients: patients with chronic pancreatitis, patients with pancreatic cancer, and a control group of healthy subjects with age and gender distributions similar to those in the first two groups.
c. Evaluation of the plasma microRNA profile in pancreatic cancer patients as compared to healthy individuals.
d. Evaluation of the cost-effectiveness of implementing these biomarkers into the long-term follow-up strategy for patients with chronic pancreatitis in relation to pancreatic cancer morbidity and mortality.

## Chapter 4. General research methodology

Study design: prospective, non-randomized study.

Selection of subjects: 60 patients of the Bucharest Clinical Emergency Hospital were enrolled, 30 with chronic pancreatitis and 30 with pancreatic cancer, over a period of 2.5 years (January 2020 – May 2022). Histopathological examination of tissue samples obtained by endoscopically guided fine aspiration biopsy (EUS-FNB) established the positive diagnosis. All participants in the study signed an informed consent form in which they consented to the processing of their personal data and the acquisition of biological samples (blood).

The ethics committee of the Bucharest Emergency Clinical Hospital approved the study (no. 3929/12.04.2021). Furthermore, an agreement (framework contract) on collaboration in research activity was established between the Bucharest Emergency Clinical Hospital and Stefan S. Nicolau Institute of Virology Bucharest (no. 560/12.04.2022), allowing the processing of biological samples within the Institute of Virology Stefan S. Nicolau.

Inclusion criteria:

- Group 1: 60 patients with a histopathological diagnosis of pancreatic cancer.
- Group 2: 60 patients with chronic pancreatitis, known to be a major risk factor for the development of pancreatic cancer.
- Group 3: 10 healthy subjects, with an age and gender distribution similar to that of the patients in the first two groups.

Exclusion criteria:

- Refusal to sign informed consent regarding participation in the study.
- patients who have associated malignancies with another location, to reduce error rates.
- patients in whom a series of paraclinical variables followed in the study could not be identified.
- patients with inadequate (hemolyzed) blood samples, which did not allow the subsequent correct processing.
- patients for whom insufficient blood could not be drawn (dehydrated or undergoing chemotherapy).
- patients in whom the amount of plasma was not sufficient for the biological and molecular determinations included in the research methodology.

For the statistical analysis of the epidemiological, clinical, and paraclinical data, we used the R program with the following packages loaded: effects, ggplot2, ggpubr, gtsummary, and logisticRR. We set the level of significance α at 0.05, so values $p < 0.05$ were considered statistically significant. For the statistical analysis of the proteomic study's results, we utilized GraphPad-Prism 9.0.

**Chapter 5. Clinical and biological data in patients with pancreatic cancer versus chronic pancreatitis**

**Introduction.** Despite advances in imaging diagnosis, differentiating pancreatic ductal adenocarcinoma from chronic pancreatitis remains a challenge [14]. Chronic pancreatitis can manifest as a tumor mass on cross-sectional imaging and is a significant risk factor for pancreatic cancer [14]. Differentiating between the two conditions is essential for evaluating patients' prognosis and establishing optimal therapeutic management. In some cases, histopathological examination of the surgical resection specimen revealed an initial misdiagnosis between benign and malignant pancreatic lesions [14]. In light of the poor prognosis of pancreatic cancer patients, which is primarily attributable to late diagnosis in therapeutically advanced stages, we proposed to conduct a study whose primary objective was to identify clinical and paraclinical factors that would aid in differentiating between chronic pancreatitis and pancreatic cancer. Avoiding a diagnostic confusion between the two conditions would enable avoiding a delay in the diagnosis of pancreatic cancer. Early detection of this malignancy is extremely essential because it can increase the percentage of patients who can lend themselves to surgical treatment, the only therapeutic method with curative potential, currently.

**Material and method.** We conducted a prospective, observational, non-randomized study in which 120 patients of the Bucharest Clinical Emergency Hospital were enrolled, over a period of 2.5 years.

The inclusion criteria were represented by:

Group 1: patients with pancreatic cancer in stages III–IV.

Group 2: patients with chronic pancreatitis.

The exclusion criteria were represented by:

- the presence, at the time of enrollment, of a personal pathological history of cancer at another site or synchronous malignancies (to eliminate certain error factors).
- lack of informed consent.
- patients whose medical documents had filling errors.

*Statistical analysis*. The collected data were centralized in a Microsoft Excel database. Later, for the statistical analysis, we used the R program with the following packages loaded: effects, ggplot2, ggpubr, gtsummary, and logisticRR. The significance level α was 0.05, so p-values lower than 0.05 were considered statistically significant. The Gaussian distribution was checked using the Shapiro-Wilk test and quantile-quantile plots (qq plots).

**Results and discussion.** The relative risk ratio of pancreatic cancer versus chronic pancreatitis was found to be twice as high in females compared to males (95% CI 0.27-0.83) and increasing patient age by 1 year resulted in a 12% increase in the likelihood of developing pancreatic cancer (95% CI 2.94 - 31.2). These outcomes align with those reported in the specialized literature. Wang *et al.* report a risk ratio of 4.5 for chronic pancreatitis between men and women [15]. Estradiol has been implicated in the pathogenesis of chronic pancreatitis in animal studies [16]. Consequently, estradiol treatment appears to inhibit acinar cell apoptosis, regardless of T cell-mediated alterations or corticosterone or testosterone levels [16]. In regards to body mass index (BMI), alcohol consumption, and smoking, there were no statistically significant differences between the two patient groups. The fact that smoking and alcohol consumption are both recognized risk factors for both conditions can help to explain this. Also, patients with chronic pancreatitis usually develop malabsorption syndromes as a result of exocrine pancreatic insufficiency and chronic inflammation, and patients with pancreatic cancer associate consumptive syndrome with weight loss. Regarding personal pathological and hereditary history, our investigation identified only one correlation with statistical significance, namely between the history of acute pancreatitis and the risk of progression to chronic pancreatitis ($p<0.001$). In the second stage of this study, we analyzed a series of biological parameters, usually determined

routinely at the first visit to the doctor. Thus, we identified, among patients with pancreatic cancer, higher values of aspartate aminotransferase (p=0.005), alanine aminotransferase (p=0.006), total bilirubin (p<0.001), direct bilirubin (p<0.001), alkaline phosphatase (p 0.030), C-reactive protein (p 0.049) and uric acid (p 0.001) [8]. In contrast, patients with chronic pancreatitis, compared to those with pancreatic cancer, had higher values of amylase (p 0.020) and lipase (p 0.029) [8]. The tumor markers carbohydrate antigen (CA19-9) and carcinoembryonic antigen (CEA) were also investigated. However, only a limited number of patients had these markers determined, so the differences were not statistically significant. However, according to our study, individuals diagnosed with pancreatic cancer exhibited nearly twice the amount of CEA compared to those diagnosed with chronic pancreatitis and approximately 70 times the amount of CA-19-9.

## Chapter 6. The role of proteomic analysis in the early diagnosis of pancreatic cancer

**Introduction.** In order to improve the prognosis of patients with pancreatic cancer, research in recent years has focused on the identification of biomarkers that may play a role in the early diagnosis of this neoplasia. Advances in molecular diagnostics, such as the detection of proteins, free circulating DNA, or microRNA, have shown promise for early pancreatic cancer identification [7]. An important advantage of these molecular techniques is their non-invasive nature [7].

The aim of our research is to identify potential non-invasive biomarkers that could aid in the early detection of pancreatic cancer. We thus performed a screening proteomic analysis on plasma pools from patients with pancreatic cancer versus patients with chronic pancreatitis using a multianalyte kit. Following this analysis, we identified a single plasma protein with significantly increased values in patients with pancreatic cancer versus chronic pancreatitis, namely leptin. Subsequently, we sought to validate these results by quantitatively determining leptin concentrations in individual plasma samples using the ELISA technique. The evidence regarding the potential role of CEACAM1 in the early detection of pancreatic cancer, as well as the positive correlation between this biomarker and body mass index, similar to that of leptin, prompted us to evaluate leptin and CEACAM1 expression simultaneously [17,18]. Furthermore, CEACAM1 appears to regulate leptin expression by interfering with certain signaling pathways also involved in oncogenesis.

**Material and method.** We conducted a prospective, experimental study lasting 2.5 years in which we defined three groups of subjects: group 1, consisting of 30 patients with

pancreatic cancer; group 2, consisting of 30 patients with chronic pancreatitis; and group 3, consisting of 10 healthy subjects, distributed in age and gender groups similar to the patients in the first two groups. The ethics committee of the Emergency Clinical Hospital of Bucharest approved this study (no. 3929/12.04.2021), and it followed the principles of the Declaration of Helsinki. All subjects included in the study signed an informed consent form in which they agreed to the processing of personal data and the collection of blood samples. The positive diagnosis was established following the histopathological examination. Biological samples were obtained by EUS-FNB.

From each subject included in the study, we collected 6 ml of blood (each in 2 ethylenediamine tetraacetic acid (EDTA) -coated test tubes). We transported the blood samples, in a maximum of two hours, to the Stefan S. Nicolau Institute of Virology, where we centrifuged them to isolate plasma (two Eppendorf tubes with 1.5 ml of plasma from each patient). Afterwards, we stored the plasma samples in freezers at -80 degrees Celsius.

*Comparative proteomic analysis of plasma pools in the three groups of patients*. In the screening stage of the proteomic analysis, we used the Proteome Profiler Human XL Cytokine Array Kit (ARY022B, R&D Systems, Abingdon, UK) based on the dot-blot technique for the simultaneous semi-quantitative determination of 105 analytes (cytokines, chemokines, angiogenesis markers, growth factors, and other soluble proteins) in pooled plasma samples from the three categories of subjects.

*Quantitative determination of leptin and CEACAM1 plasma concentrations using the ELISA technique.* To validate the results on individual samples, we used the Invitrogen KAC2281 and ab215540 Human CEACAM1 Simple Stept ELISA kits.

**Results and discussion.** Statistical analysis identified a relative similarity between plasma protein expression in patients with pancreatic cancer and those with chronic pancreatitis compared to healthy subjects. Thus, the proteins with increased expression in both categories of patients were: apolipoproteins A-I, B-cell activating factor (BAFF), brain-derived neurotrophic factor (BDNF), complement components C5/C5a, CD 40 /TNFSF5 ligand, granulocyte colony stimulating factor (G-CSF), growth differentiation factor 15 (GDF-15), insulin like growth factor binding protein 3 (IGFBP-3), IL-1alfa (IL-1F1), vitamina D BP, T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), urokinase-type plasminogen activator receptor (uPAR), myeloperoxidases, MIG, matrix metallopeptidase 9 (MMP-9), platelet-derived growth factor AA (PDGF-AA), platelet-derived growth factor AB/BB (PDGF AB/BB), pentraxina 3/TSG-14, suppression of tumorigenicity 2 protein ST2 (IL2 R4) and trefoil factor 3 (TFF3). A comparison of plasma

protein expression in pancreatic cancer patients vs. chronic pancreatitis patients revealed just one protein with enhanced expression, leptin (x 6.3 FC). We validated these results by using the ELISA technique on individual plasma samples, proving higher plasma concentrations of leptin in patients with pancreatic cancer versus those with chronic pancreatitis (59.41 ng/ml vs. 17.96 ng/ml; p = 0.0405). This difference remained statistically significant, independent of other factors such as BMI, sex, age, smoking, alcohol consumption, history of diabetes, serum triglyceride levels, and cholesterol levels.

To strengthen these results, we continued our experiments with the quantitative ELISA determination of CEACAM1. The reason that led us to determine CEACAM1 plasmatic expression was represented by the evidence of its involvement not only in pancreatic oncogenesis but also in the regulation of leptin expression. We thus identified statistically significantly higher CEACAM1 plasma values in patients with pancreatic cancer compared to those with chronic pancreatitis (12,75 ng/ml versus 9,89 ng/ml, p = 0,0282). However, the results regarding the correlation between the plasma expression of the two biomarkers were not statistically significant.

Following that, we tried to find other factors that can influence the expression of leptin and CEACAM1 in the two patient groups using simple and later multiple linear regression analysis. Thus, we detected a rise in plasma leptin levels that was independent of age, gender, BMI, smoking, alcohol intake, diabetes history, serum triglyceride and cholesterol levels. In contrast, CEACAM1 plasma concentration was found to be dependent on multiple parameters, such as the diagnosis of pancreatic cancer or chronic pancreatitis (p 0.029, 95%CI 24.67 – 460.1), gender (p 0.001, 95%CI -588.1 - - 157.3) BMI (p 0.0077, 95%CI 8.73 – 54.27) and serum triglyceride level (p 0.0002, 95%CI 1.11 – 3.39). We can thus explain the lack of correlation between the plasma expression of the two biomarkers, despite the higher serum levels of both, in pancreatic cancer patients. In order to detect the early malignant transformation of pancreatic lesions, we also hypothesize that monitoring serum leptin concentrations in patients with chronic pancreatitis may be valuable.

Leptin is an adipokine that regulates appetite, lipid metabolism, glucose homeostasis, and body weight [19,20]. It is encoded by the LEP gene and synthesized in adipose tissue [19,20]. Ob-R, a transmembrane receptor, mediates the action of leptin [20]. Multiple gastrointestinal tissues and tumor cell lines have been found to express the Ob-R receptor [21,22]. Moreover, certain malignancies may express both leptin and the Ob-R receptor, allowing autocrine signaling [23]. Effects such as the promotion of proliferation, migration, cell invasion, and tumor angiogenesis or the inhibition of tumor cell apoptosis can explain

the role of leptin in oncogenesis [22]. In addition, leptin can inhibit the activity of regulatory T cells, thereby influencing the immune surveillance process for gastrointestinal malignancies [24]. In the PhD thesis, the signaling pathways that leptin interferes with during the oncogenesis process are described in depth.

CEACAM molecules are found on the surface of numerous cell types, including epithelial and endothelial cells [25]. These molecules are involved in the modulation of the cell cycle and cell adhesion, angiogenesis, intercellular and intracellular signaling, inflammatory processes, tumor progression, and metastasis [25]. CEACAM1 can regulate cell signaling by acting as a molecular sensor at the cell surface [25]. This biomarker is also a ligand of the insulin receptor, and its activation by this receptor's tyrosine kinase initiates a cascade of signaling pathways implicated in cell growth and proliferation [26]. Wei et al. reported that the DcR3 receptor is overexpressed in the plasma of pancreatic cancer patients, as well as in tumor tissue [27]. DcR3 has been linked to an increase in signal transducers and activators of transcription (STAT1) phosphorylation and, secondarily, an increase in the expression and activity of interferon regulatory factor 1 (IRF1) [27]. DcR3/STAT1/IRF1 generated a positive feedback loop that increased CEACAM1 transcriptional activity in pancreatic cancer [27]. Recently, Pinkert *et al.* demonstrated the role of CEACAM6 in inhibiting antitumor responses such as T-cell-mediated cell death and cytokine secretion [28]. CEACAM6 exerts its immunosuppressive effect by binding to CEACAM1 on activated T cells [28].

Our study concludes that the detection of elevated serum leptin levels in patients with chronic pancreatitis should increase the suspicion of malignant transformation and expand the diagnostic management. The final aim is the early detection of pancreatic cancer, which allows for curative surgical treatment.

## Chapter 7. The role of microRNA in the early diagnosis of pancreatic cancer

**Introduction.** MicroRNAs are short RNA molecules of 19–24 nucleotides in sequence that play an important role in post-transcriptional gene expression [29]. Due to the aggressive character of pancreatic malignant neoplasms and the lack of diagnostic biomarkers, microRNAs have been studied as a promising tool for the development of predictive scores and the improvement of the therapeutic management of these patients [30,31]. In addition, microRNA-mediated intercellular communication in pancreatic cancer is poorly understood [29,32]. Restoring the levels of tumor suppressor microRNAs and inhibiting the levels of oncogenic microRNAs in healthy tissue may be useful for the maintenance of endogenous

anti-tumor regulatory mechanisms [29,32]. Despite immense progress in this field, the use of microRNAs as therapeutic targets in pancreatic cancer is hampered by significant limitations [29,32]. Lack of knowledge regarding the potential effects and toxicity levels of these chemically modified molecules on healthy cells is one of the most essential [29,32]. In conclusion, pancreatic cancer continues to be a leading cause of death worldwide, and the function of microRNAs in the management of these patients offers hope for an improvement in their prognosis.

Given the paucity of studies comparing the plasma expression of microRNAs in pancreatic cancer patients to healthy subjects, we chose this topic as the primary focus of the following investigation. Thus, we established two study groups: group 1 consisted of 23 patients with pancreatic cancer, and group 2 consisted of 10 healthy subjects with similar age and gender distributions to the group 1 patients. Using the real-time PCR technique, we initially performed a screening study in plasma pools from the two study groups for a panel of 176 microRNAs. Following this step, we identified 23 microRNAs overexpressed in pancreatic cancer patients versus the control group, results that we subsequently validated through quantitative determinations in individual plasma samples.

**Material and method.** We conducted an experimental study in which we included 23 patients with pancreatic cancer in stages III or IV of the disease and 10 healthy subjects distributed in similar age and sex groups. From each patient, we collected 6 ml of blood in EDTA-coated test tubes, which we transported in a maximum of two hours to the Stefan S. Nicolau Institute of Virology, Bucharest. Prior to inclusion in the study, each patient signed an informed consent form agreeing to the use of their personal data and the collection of blood samples for genomic analysis.

In the first step, we centrifuged the samples and isolated plasma in Eppendorf tubes, which we stored at -80 degrees Celsius. In the second step, we performed a screening analysis for 176 microRNAs in pancreatic cancer patients versus control subjects in plasma pools. The micro-RNAs identified at this stage as having elevated expression in pancreatic cancer patients compared to healthy subjects were subsequently evaluated in individual plasma samples using real-time PCR.

The data were entered into a Microsoft Excel database, and GraphPad Prism 9 was used for statistical analysis.

**Results and discussion.** Following the screening stage, we identified the overexpression of 23 microRNA in pancreatic cancer patients compared to healthy individuals. We validated the overexpression of miR-34a-5p, miR-100-5p, miR-193-5p,

miR-122-5p, and miR-885-5p in individual plasma samples during the second phase of the study. Using the Mann-Whitney test, we identified a statistically significant increase for all five of these microRNAs in the plasma of pancreatic cancer patients compared to healthy controls. In order to identify other factors that may influence the expression of these microRNAs in pancreatic cancer patients, we continued the statistical analysis with a simple linear regression, in which the dependent variable was the microRNA plasma concentration and the independent variables were tumor stage, smoking, alcohol consumption, the history of acute or chronic pancreatitis, diabetes, as well as the plasma concentrations of leptin and CEACAM1, determined by the previous study. Thus, we identified a single factor that influenced the expression variation of miR-34a-5p, miR-100-5p, miR-193-5p, miR-122-5p, and miR-885-5p in patients with pancreatic cancer, namely the tumor stage. As a result, we highlight not only the overexpression of these five microRNAs in pancreatic cancer patients but also their prognostic role.

However, future studies on larger cohorts of patients are needed to validate these results. We also emphasize the need to evaluate the expression of these microRNAs in patients with early-stage pancreatic cancer or premalignant lesions.

## Chapter 8. Conclusions and personal contributions

In my PhD research, I identified a series of clinical parameters and biomarkers that can aid in the early detection of pancreatic cancer, thereby attaining the intended goals.

In the first stage of my PhD research, we performed a prospective, observational study in which we sought to identify some clinical and paraclinical markers, evaluated routinely, that would contribute to the differential diagnosis between chronic pancreatitis and pancreatic cancer, thus avoiding delays in the detection of malignant transformation. Thus, we identified in patients with pancreatic cancer, compared to those with chronic pancreatitis, higher serum values of transaminases, total bilirubin, direct bilirubin, alkaline phosphatase, C-reactive protein, and uric acid. Also, in this subpopulation of patients, the female gender ($p = 0.001$) and age over 60 years (p 0.001) dominated. In contrast, patients with chronic pancreatitis, compared to those with pancreatic cancer, showed slightly higher values of serum amylase and lipase. In light of the fact that all of these parameters are routinely evaluated during the initial doctor visit, a meticulous analysis can guide the subsequent diagnostic management towards one of the two conditions.

In the second stage of my PhD research, I identified leptin overexpression in pancreatic cancer by analyzing the plasma expression of 105 cytokines, chemokines, growth factors,

angiogenesis markers, and other soluble proteins in pancreatic cancer patients, chronic pancreatitis patients, and healthy subjects. We validated these results by determining the concentration of leptin in individual plasma samples using the ELISA technique. We thus identified higher plasma values of this biomarker in patients with pancreatic cancer compared to those with chronic pancreatitis, the difference being statistically significant.

CEACAM1 is another biomarker that has been implicated in pancreatic oncogenesis. In addition, CEACAM1 and leptin inhibit the activity of T cells and NK cells, influencing the immune surveillance process. Our study also demonstrates higher concentrations of CEACAM1 in patients with pancreatic cancer compared to those with chronic pancreatitis. However, CEACAM 1 plasma expression was found to be dependent on several parameters, namely, diagnosis of pancreatic cancer versus chronic pancreatitis, gender, BMI, and serum triglyceride level. Thus, we can explain the absence of a correlation between the variation in CEACAM1 plasma expression and the variation in leptin plasma expression (which is dependent solely on the diagnosis). All of these findings support the hypothesis that leptin can be useful for differentiating between the two conditions. We thus suggest the effectiveness of monitoring serum leptin levels in patients with chronic pancreatitis in order to identify malignant transformation.

The originality of our investigation derives from the comparison of plasma concentrations of leptin and CEACAM1 in patients with pancreatic cancer versus patients with chronic pancreatitis. Currently, there is data supporting the involvement of these two biomarkers in pancreatic carcinogenesis, but their role in differentiating pancreatic cancer from chronic pancreatitis is unknown. The limitation of our study is that all patients with pancreatic cancer presented with stage III or IV disease. Thus, future studies evaluating the variation of serum leptin and CEACAM1 concentrations between chronic pancreatitis and early stages of pancreatic cancer are needed. Also, another limitation of our research is the relatively small size of the study cohort. A well-known challenge facing research in this field is the low incidence of pancreatic cancer and the need for national and international collaborations to obtain a sufficient number of biological samples needed to identify new biomarkers.

In the last part of the research, we conducted an experiment in which I screened plasma pools for 176 microRNAs using the real-time PCR technique. Twenty-three of these were found to be overexpressed in pancreatic cancer patients relative to healthy controls. We subsequently validated the overexpression of miR-34a-5p, miR-100-5p, miR-193-5p, miR-122-5p, and miR-885-5p in individual plasma samples. We thus identified, by using the

Mann-Whitney test, a statistically significant increase for all five microRNAs in the plasma of pancreatic cancer patients compared to control subjects. In addition, using simple regression analysis, we identified a single factor that influenced the expression variation of miR-34a-5p, miR-100-5p, miR-193-5p, miR-122-5p, and miR-885-5p in pancreatic cancer patients: tumor stage. We thus emphasize not only the overexpression of these five microRNAs in pancreatic cancer patients but also their prognostic role.

In conclusion, we identified two proteomic markers that may contribute to the detection of pancreatic cancer among patients with chronic pancreatitis, namely leptin and CEACAM-1. We also validated five microRNAs with increased expression in pancreatic cancer patients compared to control subjects, namely miR-34a-5p, miR-100-5p, miR-193-5p, miR-122-5p, and miR-885-5p. Future research is required to elucidate the mechanisms by which these biomarkers contribute to pancreatic oncogenesis. In addition to their diagnostic function, these biomarkers may represent prospective therapeutic targets for the development of new therapies to reduce the risk of malignant transformation of benign pancreatic diseases or for the treatment of pancreatic cancer.

**References**


1. Eissa MAL, Lerner L, Abdelfatah E, Shankar N, Canner JK, Hasan NM, Yaghoobi V, Huang B, Kerner Z, Takaesu F, et al. Promoter methylation of ADAMTS1 and BNC1 as potential biomarkers for early detection of pancreatic cancer in blood. Clin Epigenetics, 11, 59, 2019.
2. van Roessel S, Kasumova GG, Verheij J, Najarian RM, Maggino L, de Pastena M, Malleo G, Marchegiani G, Salvia R, et al. International validation of the eighth edition of the American Joint Committee on Cancer (AJCC) TNM staging system in patients with resected pancreatic cancer. JAMA Surg, 153, e183617, 2018.
3. Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, Hruban RH. Recent progress in pancreatic cancer. CA Cancer J Clin, 63, 318-348, 2013
4. Lennon AM,Wolfgang CL, Canto MI, Klein AP, Herman JM, Goggins M, Fishman EK, Kamel I, Weiss MJ, Diaz LA, et al. The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? Cancer Res, 74, 3381-3389, 2014
5. Sawhney MS, Calderwood AH, Thosani NC, Rebbeck TR, Wani S, Canto MI, Fishman DS, Golan T, Hidalgo M, Kwon RS, et al. ASGE Standards of Practice Committee. ASGE guideline on screening for pancreatic cancer in individuals with genetic

susceptibility: summary and recommendations. Gastrointestinal Endoscopy, 95, 817-826, 2022.

6. Kaur S, Baine MJ, Jain M, Sasson AR, Batra SK. Early diagnosis of pancreatic cancer: challenges and new developments. Biomark Med, 6, 597-612, 2012.
7. Gheorghe G, Bungau S, Ilie M, Behl T, Vesa CM, Brisc C, Bacalbasa N, Turi V, Costache RS, Diaconu CC. Early diagnosis of pancreatic cancer: the key for survival. Diagnostics, 10, 869, 2020.
8. Gheorghe G, Ionescu VA, Moldovan H, Diaconu CC. Clinical and biological data in patients with pancreatic cancer vs. chronic pancreatitis – a single center comparative analysis. Diagnostics, 13, 369, 2023.
9. Diaconu CC, Gheorghe G, Hortopan A, Enache V, Ceobanu G, Jinga V, Adrian C, Ionescu VA. Pancreatic tuberculosis- a condition that mimics pancreatic cancer. Medicina, 58, 1165, 2022.
10. Pereira SP, Oldfield L, Ney A, Hart PA, Keane MG, Pandol SJ, Li D, Greenhalf W, Jeon CY, Koay EJ, Almario CV, Halloran C, Lennon AM, Costello E. Early detection of pancreatic cancer. Lancet Gastroenterol Hepatol, 5, 698-710, 2021.
11. Ansari D, Torén W, Zhou Q, Hu D, Andersson R. Proteomic and genomic profiling of pancreatic cancer. Cell Biol. Toxicol, 35, 333–343, 2019.
12. Soreide K, Ismail W, Roalso M, Ghotbi J, Zaharia C. Early Diagnosis of Pancreatic cancer: clinical premonitions, timely precursor detection and increased curative-intent surgery. Cancer Control, 30, 10732748231154711, 2023.
13. Singhi AD, Koay EJ, Chari ST, Maitra A. Early detection of pancreatic cancer: opportunities and challenges. Pancreatic Cancer, 156, 2024-2040, 2019.
14. Hamsen FJ, Domagk D, Dietrich CF, Hocke M. Discriminating chronic pancreatitis from pancreatic cancer: contrast enhanced EUS and multidetector computed tomography in direct comparision. Endosc Ultrasound, 7, 395-403, 2018.
15. Wang M, Gorelick F, Bhargava A. Sex differences in the exocrine pancreas and associated diseases. Cell Mol Gastroenterol Hepatol, 12, 427-441, 2021.
16. Nakamura S, Yamada T, Hashimoto T, Takahashi S, Sogawa M, Ohara H, Nakazawa T, Sano H, Kuno A, Joh T, Nomura T, Arakawa T, Itoh M. Estradiol alleviates acinar cell apoptosis and chronic pancreatitis in male Wistar Bonn/Kobori rats. Pancreas, 26, e59–e66, 2003.
17. Simeone SM, Ji B, Banerjee M, Arumugam T, Li D, Anderson M, Bamberger AM, Greenson J, Brand R, Ramachandran V, et al. CEACAM1, a novel serum biomarker for

pancreatic cancer. Pancreas, 34, 436-443, 2007.
18. Najjar S, Russo L. CEACAM1 loss links inflammation to Insulin Resistance in obesity and Non-alcoholic Steatohepatitis (NASH). Semin Immunopathol, 36, 55-71, 2013.
19. Stolzenberg-Solomon RZ, Newton CC, Silverman DT, Pollak M, Nogueira LM, Weinstein SJ, Albanes D, Mannisto S, Jacobs EJ. Circulating Leptin and Risk of Pancreatic Cancer: A Pooled Analysis From 3 Cohorts. Am J Epidemiol, 182, 187-197, 2015.
20. Marroquí L, Gonzalez A, Ñeco P, Caballero-Garrido E, Vieira E, Ripoll C, Nadal A, Quesada I. Role of leptin in the pancreatic β-cell: effects and signaling pathways. J Mol Endocrinol, 491, R9–R17, 2012.
21. Chan JL, Blüher S, Yannakouris N, Suchard MA, Kratzsch J, Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes, 51, 2105-12, 2002.
22. Alemán JO, Eusebi LH, Ricciardiello L, Patidar K, Sanyal AJ, Holt PR. Mechanisms of obesity-induced gastrointestinal neoplasia. Gastroenterology, 1462, 357–373, 2002.
23. Sennello JA, Fayad R, Morris AM, Eckel RH, Asilmaz E, Montez J, Friedman JM, Dinarello CA, Fantuzzi G. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. Endocrinology, 146, 2157–64, 2005.
24. Hoda MR, Keely SJ, Bertelsen LS, Junger WG, Dharmasena D, Barrett KE. Leptin acts as a mitogenic and antiapoptotic factor for colonic cancer cells. Br J Surg, 94, 346–54, 2007.
25. Zinczuk J, Zareba K, Romaniuk W, Kaminska D, Niziol M, Baszun M, Kedra B, Guzinska-Ustymowicz K, Pryczynicz A. Expression of Chosen Carcinoembryonic-Related Cell Adhesion Molecules in Pancreatic Intraepithelial Neoplasia (PanIN) Associated with Chronic Pancreatitis and Pancreatic Ductal Adenocarcinoma (PDAC). Int J Med Sci, 16, 583-592, 2019.
26. Ling Y, Wang J, Wang L, Hou J, Qian P, Xiang-Dong W. Roles of ceacam1 in cell communication and signaling of lung cancer and other diseases. Cancer Metastasis Rev, 34, 347–57, 2015.
27. Wei Y, Chen X, Yang J, Yao J, Yin N, Zhang Z, Li D, Zhu D, Zhou J. DcR3 promotes proliferation and invasion of pancreatic cancer via a DcR3/STAT1/IRF1 feedback loop. Am J cancer Res, 9, 2618-2633, 2019.
28. Pinkert J, Boehm HH, Trautwein M, Doecke WD, Wessel F, Ge Y, Gutierrez EM, Carretero R, Freiberg C, Gritzan U, et al. T cell-mediated elimination of cancer cells by

blocking CEACAM6–CEACAM1 interaction. Oncoimmunology, 11, 2008110, 2022.

29. Rawat M, Kadian K, Gupta Y, Kumar A, Chain PSG, Kovbasnjuk O, Kumar S, Parasher G. MicroRNA in pancreatic cancer: from biology to therapeutic potential. Genes, 10, 752, 2019.
30. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 75, 843–854, 1993.
31. Daoud AZ, Mulholland EJ, Cole G, McCarthy HO. MicroRNAs in pancreatic cancer: biomarkers, prognostic and therapeutic modulators. BMC Cancer, 19, 1130, 2019.
32. Li Y, Sarkar FH. MicroRNA targeted therapeutic approach for pancreatic cancer. Int J Biol Sci, 12, 326–337, 2016.

## List of published scientific papers

### Articles published in ISI Clarivate Analytics-indexed journals with an impact factor

1. **Gheorghe G**, Bungau S, Ilie M, Behl T, Vesa CM, Brisc C, Bacalbasa N, Turi V, Costache RS, Diaconu CC. Early diagnosis of pancreatic cancer: the key for survival. Diagnostics, 10(11), 869, 2020. https://doi.org/10.3390%2Fdiagnostics10110869. IF 3.6. (Chapter 2: Pancreatic cancer diagnosis, page 11-25).
2. **Gheorghe G**, Bacalbasa N, Ceobanu G, Ilie M, Enache V, Constantinescu G, Bungau SG, Diaconu C. Gastrointestinal Stromal Tumors – a mini Review. Journal of Personalized Medicine, 11(8), 694, 2021. https://doi.org/10.3390%2Fjpm11080694. IF 3.508. (Chapter 1: Epidemiological and etiopathogenic data, page 1-10; Chapter 2: Pancreatic cancer diagnosis page 11-25).
3. **Gheorghe G**, Diaconu CC, Ionescu V, Constantinescu G, Bacalbasa N, Bungau S, Gaman MA, Stan-Ilie M. Risk factors for pancreatic cancer: emerging role of viral hepatitis. J Pers Med, 12(1), 83, 2022. https://doi.org/10.3390/jpm12010083. IF 3.508. (Chapter 1: Epidemiological and etiopathogenic data, page 1-10).
4. Diaconu CC, **Gheorghe G,** Hortopan A, Enache V, Ceobanu G, Jinga V, Adrian C, Ionescu VA. Pancreatic tuberculosis- a condition that mimics pancreatic cancer. Medicina, 58(9), 1165, 2022. https://doi.org/10.3390/medicina58091165. IF 2.948. Corresponding author. (Chapter 5: Clinical and biological data in patients with pancreatic cancer versus chronic pancreatitis, page 41-56).
5. **Gheorghe G**, Ionescu VA, Moldovan H, Diaconu CC. Clinical and biological data in patients with pancreatic cancer vs. chronic pancreatitis – a single center comparative analysis. Diagnostics, 13(3), 369, 2023. https://doi.org/10.3390/diagnostics13030369. IF 3.992. IF 3.992. (Chapter 5: Clinical and biological data in patients with pancreatic cancer versus chronic pancreatitis, page 41-56).
6. **Gheorghe G**, Diaconu CC, Mambet C, Bleotu C, Ionescu VA, Diaconu CC. Comparative Analysis of Leptin and Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1 Plasma Expression in Pancreatic Cancer and Chronic Pancreatitis Patients. (Chapter 6: The role of proteomic analysis in the early diagnosis of pancreatic cancer, page 57-96)

**Articles published in ISI Clarivate Analytics-indexed journals without an impact factor**

7. Ionescu VA, **Gheorghe G**, Oprita R, Ilie M, Dascalu RI, Zaharia O, Jinga V, Diaconu CC, Constantinescu G. The outcomes of nutritional support techniques in patients with gastrointestinal cancers. Gastroenterology Insights, 13(3), 145-257, 2022. https://doi.org/10.3390/gastroent13030025. Corresponding author. (Chapter 5: Clinical and biological data in patients with pancreatic cancer versus chronic pancreatitis, page 41-56).

**Articles published in journals indexed in other BDI**

8. Ionescu VA, Gherghiceanu F, Gheorghe F, **Gheorghe G**. Initial approach to the patients with abdominal pain. Annals of the Academy of Romanian Scientists. Series on Medical Sciences, 3(1), 21, 2022. https://doi.org/10.56082/annalsarscimed.2022.1.21. (Chapter 2: Pancreatic cancer diagnosis, page 11-25).
9. Ionescu VA, **Gheorghe G**, Constantinescu G, Sandru V, Ilie M, Oprita R, Plotogea O, Constantinescu A, Gherghiceanu F, Diaconu C. Endoscopic radiofrequency ablation in biliopancreatic tumours. Archives of the Balkan Medical Union 56(4), 488-493, 2021. https://doi.org/10.31688/ABMU.2021.56.4.13. (Chapter 2: Pancreatic cancer diagnosis, page 11-25; Chapter 5: Clinical and biological data in patients with pancreatic cancer versus chronic pancreatitis, page 41-56)
10. Ceobanu G, **Gheorghe G**, Ionescu VA. Intraductal papillary mucinous neoplasm of the pancreas – risk of malignancy. Annals of the Academy of Romanian Scientists. Series on Medical Sciences, 2(2), 14-20, 2021. https://doi.org/10.56082/annalsarscimed.2021.1.14. (Chapter 1: Epidemiological and etiopathogenic data, page 1-10)