

# EARLY DIAGNOSIS OF PANCREATIC CANCER BY DETERMINING GENETIC AND SEROLOGICAL TUMORAL MARKERS

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## ABSTRACT

### Introduction

Pancreatic cancer has the worst prognosis among gastrointestinal cancers. The high mortality is justified by the paucity of symptoms and by the lack of response to treatment. The lack of sensitive tumor markers specific to early diagnosis has a major contribution to the poor prognosis.

**The objective of the study** was to evaluate whether tumor markers like mesothelin, circulating tumor cells, microRNA, can be used as early diagnosis and prognostic factors in pancreatic cancer. Secondary endpoints aimed to test these markers in chronic pancreatitis in order to create a panel of markers for high-risk population screening.

### Material and methods

We measured the concentration of 3 markers (mesothelin, miR-10b and miR-155) using blood samples from the three groups: neoplasm, pancreatitis and control group (healthy) and tried to identify statistically significant correlations between them.

### Results

Pancreatic cancer can be diagnosed using blood biomarkers such as mesothelin and certain types of miR.

## RÉSUMÉ

Détermination des marqueurs tumoraux sériques pour le diagnostic précoce du cancer du pancréas

### Introduction

Le cancer pancréatique a le pire pronostic parmi ceux de l'appareil digestif. La mortalité élevée est justifiée par la pénurie de symptômes, et par une faible réponse au traitement. L'absence de marqueurs tumoraux pour le stage précoce explique le mauvais pronostic.

**L'objectif** est d'évaluer si des marqueurs comme la mésothéline, les cellules tumorales circulantes, ou microARN peuvent être utilisés pour le diagnostic précoce et comme facteurs pronostiques. L'objectif secondaire est de tester ces marqueurs dans la pancréatite chronique et les utiliser pour le dépistage parmi la population à haute risque.

### Matériel et méthodes

Nous avons mesuré la concentration sérique de mésothéline, miR-10b et miR-155 chez des échantillons appartenant aux trois catégories: groupe avec cancer du pancréas, groupe avec pancréatite et groupe-contrôle (sains) et essayé de montrer des corrélations entre eux.

There has been elevated plasma mesothelin in patients with pancreatic neoplasia and chronic pancreatitis compared to healthy patients. Mesothelin could be used for differentiation between neoplasm patients and chronic pancreatitis ( $p=0.05$ ). There was a direct correlation between tumor size and miR-10b ( $p=0.05$ ), but none between mesothelin and miR-10b ( $p=0.53$ ).

### Conclusions

A better understanding of the principles and complex mechanisms of genes expression associated to miRNA may lead to new therapy and diagnosis opportunities for the pancreatic cancer and may become the premises of a screening strategy for the patient with high risk of developing pancreatic cancer.

**Key-words:** pancreatic cancer, chronic pancreatitis, mesothelin, miR-10b, miR-155.

## INTRODUCTION

The pancreatic cancer has the worst prognosis among gastrointestinal cancers, with a mortality rate close to its incidence. The analysis on the globe carried out by GLOBOCAN in 2012 places the pancreatic cancer on the 13<sup>th</sup> place in terms of incidence and on the 8<sup>th</sup> place in terms of mortality of all cancers, and in relation with digestive cancers it occupies the 6<sup>th</sup> place for both epidemiological indices.<sup>1</sup>

Due to the lack of mesentery, because of the intimate contact with the common biliary duct and other retroperitoneal structures and to the nearby position with the stomach, duodenum and colon, most clinical manifestations represent the late consequence of the invasion or compression of these structures.<sup>2</sup> For this reason, 80-85% of the patients present in the inoperable stage.<sup>3</sup>

For the diagnosis of pancreatic cancer, the European Society of Medical Oncology (ESMO) recommends abdominal ultrasound as an initial investigation, Endoscopic Retrograde Cholangiopancreatography (ERCP) for biliary obstructions, and endoscopic ultrasound, contrast-enhanced multi-detector (MD-CT), magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP) as additional investigations. Despite the fact that it has a sensitivity superior to computed tomography (CT), positron emission tomography (PET-CT) is used only to detect metastases or to investigate the uncertain results obtained by CT.<sup>4</sup>

The abdominal ultrasound is the first diagnostic test for pancreatic cancer, as it has a sensitivity of

## Résultats

Le cancer pancréatique pourrait être diagnostiqué en utilisant des marqueurs sériques. Chez les sujets avec cancer du pancréas ou pancréatite chronique des niveaux plus élevés que chez les sujets sains ont été rapportés. La mésothéline pourrait être utilisée pour différencier les sujets avec cancer de ceux ayant une pancréatite chronique ( $p=0.05$ ). Il y avait une corrélation directe entre les dimensions tumorales et MiR-10b ( $p=0.05$ ), mais aucune corrélation entre la mésothéline et miR-10b ( $p=0.53$ ).

### Conclusions

Une meilleure compréhension des mécanismes complexes de l'expression des gènes associés au miARN pourrait déboucher sur de nouvelles possibilités thérapeutiques et diagnostiques et devenir la base d'une stratégie de dépistage pour les patients présentant un risque élevé de cancer du pancréas.

**Mots-clés:** cancer pancréatique, pancréatite chronique, mésothéline, miR-10b, miR-155.

90% and a specificity of 95% for tumors larger than 3 cm. Despite all these, this is an operator-dependent method and it cannot differentiate between cancer and chronic or autoimmune pancreatitis.<sup>5</sup>

Endoscopic ultrasound (EUS) was initially used for the confirmation and staging of the solid focal tumors seen by other imaging methods (trans-abdominal ultrasound, CT or MRI). At present, it represents the nonsurgical method having the highest sensitivity (98%) in detecting benign or malignant pancreatic formations (superior to conventional computed tomography, with a sensitivity of 86%).<sup>6</sup>

CT scan is mainly used for pancreatic cancer staging.<sup>7</sup> MRI is recommended for patients with CT contraindications (nephropathy, pregnancy, and allergy to the contrast medium) or when the CT result is uncertain, and has a sensitivity of 81-99% and specificity of 70-93%.<sup>8</sup>

Biopsy using endoscopic ultrasound is recommended only if the pancreatic lesions are ambiguous during the imaging examination. Metastases may be subject to percutaneous biopsy under CT guidance, trans-abdominal ultrasound or during endoscopic ultrasound. It has been established that endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA) is the most sensitive (75-90%), specific (94-100%) and lacked of complications (below 1%) method used in the histological diagnosis of pancreatic tumors.<sup>9</sup>

Despite the development of the diagnostic tests, the surgical techniques and the chemotherapeutic treatment, the survival rate has not improved in the

past decades. Thus, after diagnosis, the one-year survival is 24%, and 5-year survival 5%.<sup>10</sup>

The latest researches have shown that there is a latency period of 10 years since the onset of the first tumor changes until the onset of the first symptoms<sup>11</sup>, a period where the use of screening biomarkers might change the prognosis and lead to an early diagnosis. During the past three decades, more markers have been proposed for the pancreatic cancer, but no marker has been implemented in the screening strategy. Among these, there was CA19-9 carbohydrate antigen and carcinoembryonic antigen (CEA). CEA is a glycoprotein used in the clinic as a tumor marker for the diagnosis of breast cancer, stomach cancer, colorectal cancer and pancreatic cancer, having a specificity of 79% and a sensitivity of only 54%. For this reason, it may be tested in combination with CA19-9, the specificity and sensitivity in diagnosis increasing to 86%.<sup>12</sup> Despite all these, the European Group on Tumor Markers (EGTM) does not recommend it due to the falsely positive results in certain cases of non-malignant jaundice.<sup>13</sup> Markers such as MIC1 (macrophage inhibitory cytokine 1), osteopontin, tissue inhibitor of matrix metalloproteinase-1, and mesothelin genes have not demonstrated their superiority towards CA19-9, in the diagnosis of the pancreatic cancer.<sup>14</sup>

Previous studies showed that microRNA plays an important role in oncogenesis and metastatic spreading of the pancreatic cancer.<sup>15</sup>

MicroRNA are non-coding RNA fragments, having a length of 20-22 nucleotides, whose essential role is in the post-transcriptional regulation of gene expression through the degradation or repression of translation of certain specific types of RNA messenger ("target"). They determine a reduction of the quantity and activity of proteins involved in cellular processes essential for the normal functioning of the cell, such as apoptosis, differentiation and cellular cycle.<sup>16</sup>

Abnormal levels of microRNA have been encountered in cancer, autoimmune diseases, viral infections or sepsis.

It has been noticed that certain types of microRNA regulate the level of proto-oncogenes or tumor suppression genes, their expression being often modified in diverse tumor tissues and, consequently, they might be used as tumor markers.

Thus, certain micro-RNA (miR-34a, miR-124, miR-143, miR-203, miR-200, miR-146a) act as proto-oncogene inhibitors having the role of a tumor suppressor, and they will appear in small quantities in the tumor tissues, whereas other types of microRNA (21,221,192,155,10a) inhibit the tumor

suppression genes, having an increased expression at tumor level.<sup>17</sup>

The lack of sensitive tumor markers specific for the early diagnosis of the pancreatic cancer has a major contribution to the poor prognosis.

**THE OBJECTIVE OF THE STUDY** was to evaluate whether tumor markers like mesothelin, circulating tumor cells, microRNA, can be used as early diagnosis and prognostic markers in pancreatic cancer. Secondary endpoints aimed to test these markers in benign pancreatic pathology (chronic pancreatitis), in order to create a panel of markers for high-risk population screening.

## MATERIAL AND METHODS

### Studied groups

The **target population** of the study was made up of people from the general population with pancreatic masses; the population included in the study consisted of patients with pancreatic cancer and chronic pancreatitis, who were treated in the Gastroenterology Clinic of the Clinical Emergency Hospital of Bucharest, Romania.

This is a **prospective study**, that took place between 5<sup>th</sup> January 2015 - 1<sup>st</sup> January 2016, in the Gastroenterology Clinic of the Clinical Emergency Hospital of Bucharest, Romania.

The study group consisted of 64 patients, who were separated in 3 groups: one with pancreatic cancer, another group with chronic pancreatitis and the third one with healthy volunteers, without any history of pancreatic diseases, as the control group. Grouping was based on blood tests and imaging tests.

The **inclusion criteria** consisted of the known pancreatic cancer diagnosis at admission, regardless of the type of medical intervention for which the patients presented (surgery or stenting), while the **exclusion criteria** were represented by insufficient data about the patients, or the refusal to participate in the study. Informed consent was obtained before blood sampling.

The patient's demographic characteristics, clinical manifestations of the disease, and the results of biological and imaging investigations were obtained from the hospital database.

**The items followed** were:

- Demographic data: age and gender.
- Laboratory tests: amylase and lipase.
- Abdominal ultrasonography/CT.
- RT-qPCR for miR-10b and miR-155 detection.
- ELISA for mesothelin screening.

**QUALITY ASSESSMENT**

The blood samples were subsequently analyzed in the laboratories of the Institute of Virology “Ștefan Nicolau”, Bucharest, Romania. The samples were initially stored at 4-80°C, and then processed rapidly by centrifugation, followed by collecting the supernatant. After processing, they were stored at -800°C until analysis.

The mesothelin was determined using the Elisa kit protocol from Biologend, while the microRNA were determined by extracting first the RNA using a TirozolLS reagent and after that the RT-qPCR reaction for in vitro amplification of RNA after it was first revers-transcript in DNAC.

**Statistical analysis of the data**

Continuous variables were expressed as mean ± standard deviation, and those stagnating as number (percent). In case of a normal dispersion in the sample, the Student T test was used to compare the media. Comparison of the values from two dependent samples was performed using parameter t or its Wilcoxon signed rank or nonparametric equivalent.

In order to study the concomitant contribution of several factors to the occurrence of an event or the magnitude of an effect, multivariate analysis was used: multiple linear regression for continuous variables, logistic regression for dichotomic variables. The statistical analysis program SPSS 19.0 and Microsoft Excel were used for the analysis.

**RESULTS**

**Demographic characteristics**

Regarding gender distribution (Fig. 1), a slight predominance of male gender can be observed, both in pancreatic cancer (M/F = 23/11) and chronic pancreatitis (M/F = 17/3). The mean age of patients was

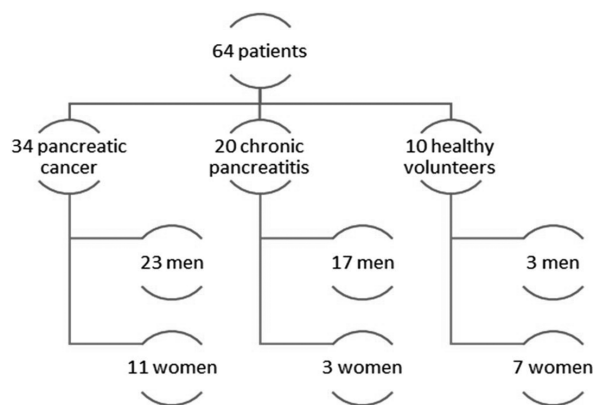


Fig. 1. Demographic features of the patients

66.02 years for patients with pancreatic cancer and 51.05 years for patients with chronic pancreatitis. If chronic pancreatitis registers a peak incidence between 40-60 years, in pancreatic cancer most patients were over 60 years of age at the time of diagnosis.

**Tumor size**

Since very few patients had TNM staging, many of whom were hospitalized for palliative treatment (stenting), they were chosen to centralize them according to the size of the tumor. The graph below (Fig. 2) shows that most of the patients (35%) had at the time of diagnosis a tumor diameter between 3-4 cm and only 15% had less than 2cm.

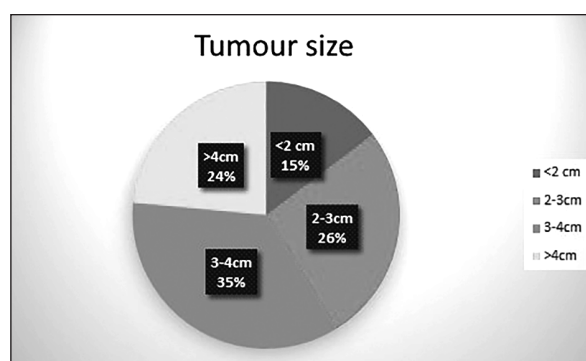


Fig. 2. Tumor size

**Common symptoms and signs in pancreatic cancer**

According to Table 1, the most common symptoms were weight loss (88.23%) and abdominal pain (85.29%). Also, 64.70% of the patients presented dark urine and 58.82% acholic stools. Nausea and vomiting were present in 44.11% of the patients, while pruritus was found in only 26.47%. (Table 1)

As signs of the disease, the most common was jaundice (73.52%), followed by the sign of Courvoisier-Terrier in 23.52% of cases, while ascites and hepatomegaly were present in less than 20% of hospitalized patients.

Table 1. Symptoms and signs in pancreatic cancer

SYMPTOMS		SIGNS	
Pruritus	26.47%	Jaundice	73.52%
Light stool color	58.82%	Courvoisier-Terrier sign	23.52%
Dark urine	64.70%	Hepatomegaly	14.70%
Nausea and vomiting	44.11%	Ascites	11.76%
Abdominal pain	85.29%		
Weight loss	88.23%		

## Plasma concentration

### Mesothelin results

Following the Elisa analysis of the samples, the optical density of the analyzed samples can be read. With these values, the graph is plotted, on a millimeter paper, on the abscissa, having the concentration of mesothelin and the absorbance on the order. To determine mesothelin concentration, a horizontal line is drawn from the mean value of each absorbent from the OY axis until it intersects the plot and the corresponding concentration value is determined from the OX axis. Below in figure 3 is an example.

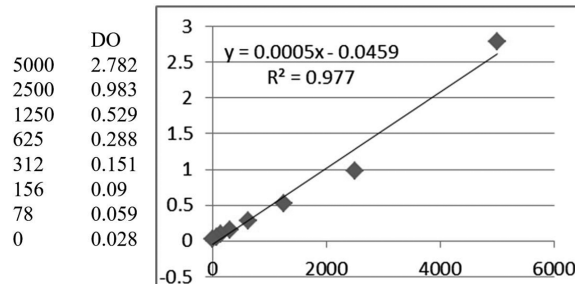


Fig. 3. ELISA analysis of the samples

### Plasma concentrations

Plasma concentrations of biomarkers obtained from the plasma of the 3 groups are shown in the following table 2. By analyzing the results, elevated plasma mesothelin in pancreatic neoplasia and pancreatitis are compared to the group of healthy patients.

For a better illustration of the distribution of these concentrations, they were plotted. (Figure 4)

### Diagnostic validity of markers

To determine the sensitivity and specificity of the markers, we used the ROC curves.

According to the figure below, mesothelin could be used for differentiation between neoplasm

patients and chronic pancreatitis (area under the curve =  $0.656 \pm 0.078$ ;  $p = 0.05$ ). Regarding the difference between pancreatitis and healthy subjects, respectively healthy neoplasms, a sufficiently large area was not obtained (AUC =  $0.49 \pm 0.11$  and AUC =  $0.656 \pm 0.09$  respectively), but the criteria of statistical significance have not been met,  $p$  exceeding 0.05 in both cases ( $p = 0.93$  and  $p = 0.13$ ).

As for miR-10b, according to the ROC curve, for our group of patients it would not have diagnostic significance (contrary to the literature), due to low values of the area under the curve, but at the same time  $p > 0.05$ . More details can be observed below in figure 5.

Table 2. Plasma levels of the markers

	Pancreatic adenocarcinoma (n=34)	Chronic pancreatitis(n=20)	Group control (n=10)	PADC vs healthy	PADC vs CP	CP vs healthy
Mesothelin	16353.65±9300.9	12660±8766.2	10102.67±3404.6	P=0.003	P=0.151	P=0.269
Min-max	5436-45556	4916-42036	6596-16756			
Mir-10b	22.56±4.23	23.54±3.97	24.9±2.89	P=0.05	P=0.39	P=0.29
Min-max	32.73-15.48	30.24-14.87	30.98-19.73			

Note: The values are presented as average  $\pm$  standard deviation, ie min-max. The statistical significance was calculated using the student *t* test. CP – Chronic pancreatitis; PADC – pancreatic adenocarcinoma.

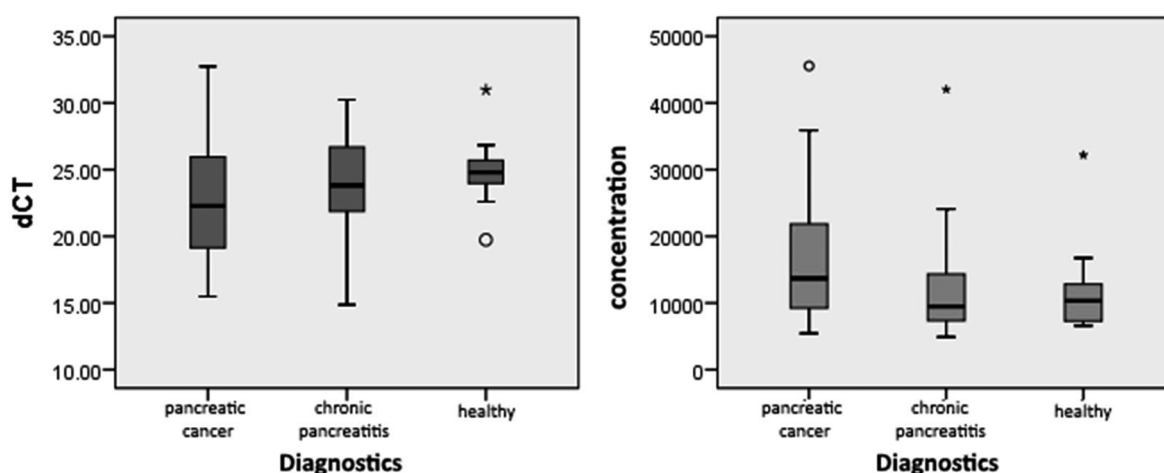


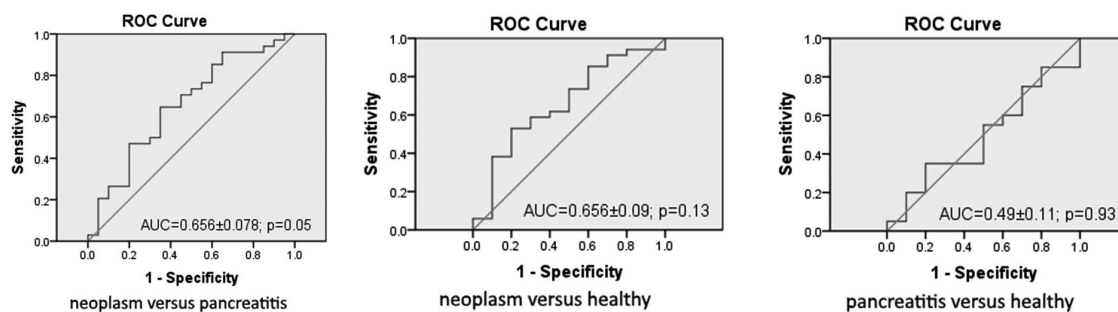
Fig. 4. Distribution of marker concentration on studied groups



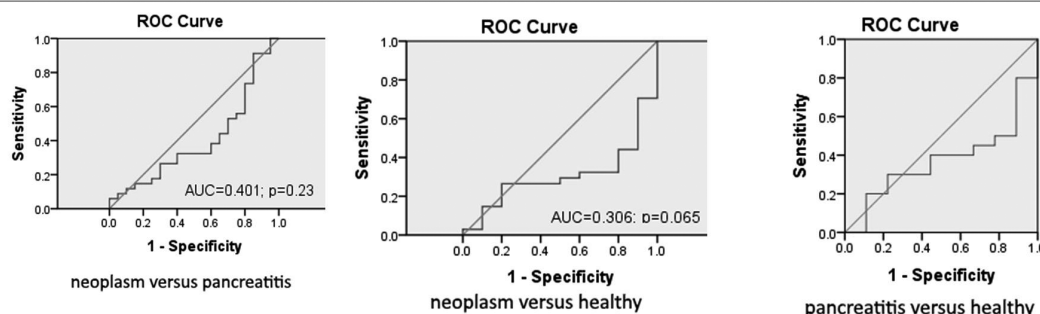
**Table 3.** Specificity and sensitivity of the markers

Marker	Neoplasm vs pancreatitis			Neoplasm vs control			Pancreatitis vs control		
	Ng/ml	Sn(%)	Sp(%)	Ng/ml	Sn(%)	Sp(%)	Ng/ml	Sn(%)	Sp(%)
Mesothelin	7796	91	65	6696	94	80	6156	90	100
	5656	97	96	6436	94	100	6636	85	90
Mir 10b	17.76	91	85	17.55	90	100	16.08	95	100
	16.50	94	95	22.56	44	90			

**Mesothelin**



**MIR10b**

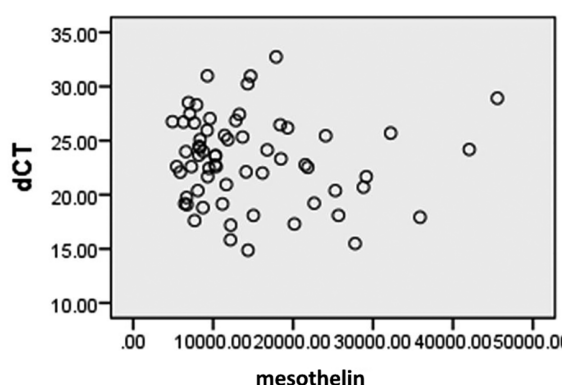


**Fig. 5.** ROC curve for mesothelin and MIR10b

In addition to calculating the area under the curve, which gives us information on the validity of the marker as a diagnostic test, the sensitivity and specificity of each of them was determined, as can be seen in the Table 3.

**Correlation between mesothelin and miR-10b**

According to the Pearson correlation coefficient, there is a very weak negative statistically insignificant correlation between mesothelin and miR-10b ( $r(62) = -.08, p = 0.53$ ). This is also supported by the random, chaotic layout of the values in the graph below without a linear distribution as is normally the case. (Table 4, Fig. 6)



**Fig. 6.** Correlation between mesothelin and miR-10b

**Correlation miR-10b – Tumor size**

The Spearman correlation index  $r(32) = 0.32$  shows that there is a statistically significant positive correlation ( $p = 0.05$ ) between the miR-10b value and the tumor size. (Table 5)

**Correlation mesothelin – tumor size**

As it can be seen in table 6, there is a very poor negative correlation between mesothelin and tumor size ( $r(32) = -0.07$  statistically insignificant ( $p = 0.69$ )). (Table 6)

**Table 4.** Correlation between miR-10b and mesothelin.

		dCT	Mesothelin
dCT	Pearson Correlation	1	-.080
	Sig. (2-tailed)		.531
	N	64	64
Mesothelin	Pearson Correlation	-.080	1
	Sig. (2-tailed)	.531	
	N	64	64

**Table 5.** The Spearman correlation between miR-10b concentration and tumor size

		size	dCT	
Spearman's rho	size	Correlation Coefficient	1.000	
		Sig. (2-tailed)	.	
		N	34	
	dCT	Correlation Coefficient	.327	1.000
		Sig. (2-tailed)	.059	.
		N	34	34

**Table 6.** Spearman correlation between mesothelin concentration and tumor size

		size	mesothelin	
Spearman's rho	size	Correlation Coefficient	1.000	
		Sig. (2-tailed)	.	
		N	34	
	mesothelin	Correlation Coefficient	-.070	1.000
		Sig. (2-tailed)	.693	.
		N	34	34

## DISCUSSION

Despite the advances in medicine in the last decade, pancreatic cancer has the worst prognosis among the digestive cancers, with mortality rates roughly equal to incidence rates. Increased mortality is explained by the shortness of symptoms, becoming clinically manifest at the time of secondary determinations, as well as the lack of response to treatment. Although nowadays we can perform imaging diagnosis through modern techniques: endoscopic ultrasound, MRI/CT and cytology, the diagnosis is often delayed after local invasion/remote metastases, with bad outcomes for the patient.

### Demographic characteristics

Regarding the age of patients at diagnosis, GLOBOCAN estimated in 2012 the highest incidence of pancreatic cancer at 65 years.<sup>1</sup> This fact was confirmed in our study, with 67% of patients being over 60 years of age.

Regarding gender distribution, there is a male/female ratio of 3/1 in the literature, slightly higher

than the ratio of patients enrolled in our study (M/F = 2.09/1).

Pancreatic ductal carcinoma is most often asymptomatic or has non-specific onset of symptomatology. The most common signs and symptoms cited in the literature are: weight loss in 92% of cases, abdominal pain 72%, dark urine and acholic stools 64%, nausea and vomiting 45%, pruritus 24%; ascites 14%, hepatomegaly 83%, jaundice 87%, the Courvoisier-Terrier sign 29%.<sup>18</sup> Compared to these data, 85.29% of the patients included in our study accused abdominal pain, 88.23% involuntarily weight loss, 44.11% had nausea or vomiting, 64.70% had dark urine, and only 24.47% had pruritus. As signs of disease, the most common was jaundice (73.52%), followed by the sign of Courvoisier-Terrier in 23.52% of cases, while ascites and hepatomegaly were present in less than 20% of hospitalized patients.

### Concentrations of markers

Due to increased incidence and high mortality, including in detectable cases of neoplasm, the discovery and establishment of a marker or a panel of

markers for early screening and early diagnosis of pancreatic cancer should be a priority in the research undertaken in this field.

It has been observed that some types of micro-RNA regulate the level of proto-oncogenes or tumor suppressor genes, their expression being often altered in various tumor tissues and therefore could be used as tumor markers.<sup>19</sup>

In our group of patients, elevated concentrations of miR-10b were observed in both neoplasm and pancreatitis as compared to those in the control group. In a more in-depth analysis, using the Student t test to compare the mean concentrations between the 3 groups (Table 2), a p-statistically significant difference ( $p = 0.05$ ) was obtained between healthy and pancreatic neoplasm. This may be due to the small size of the sample and the uneven distribution of the number of patients within the three groups.

Because in neoplasm versus pancreatitis and pancreatitis versus neoplasm the concentration differences are not statistically significant, we cannot claim that miR-10b can be used for the differential diagnosis between neoplasm and pancreatitis. As previously reported in the literature, miR-196a, miR-203, miR-210 are the only ones capable of distinguishing between neoplasm and pancreatitis.<sup>20</sup>

We observed that in the case of mesothelin, increased concentrations were obtained in the neoplasm and chronic pancreatitis groups, but the only statistically significant difference was in comparing the neoplasm group with the healthy patients ( $p = 0.03$ ). This confirms that mesothelin, the protein of the cell membranes that make up the pleura, pericardium and peritoneum, is expressed in several cancers, including mesothelioma, ovarian and pancreatic cancer, and some squamous cell carcinomas.<sup>21</sup>

MiR-155 did not express any serum samples, although it is frequently positive in pancreas biopsies. This is the case, including the laboratory test procedure.

The graphical representation of the distribution of the concentrations of the two markers expressed in the serum samples (mesothelin and miR-10b) confirms and illustrates the conclusions presented.

#### Validity of markers used

To determine the sensitivity and specificity of the markers, we used the ROC curves. Thus, the lower the value of the area below the curve is 1, the higher are sensitivity and specificity of the test. If the area is smaller, we cannot increase the sensitivity or the specificity, except at the price of an increasingly drastic decrease of the other parameter.

According to the analysis of the ROC curves, we can say that mesothelin could be used for

differentiation between neoplasm and chronic pancreatitis (area under the curve =  $0.656 \pm 0.078$ ,  $p = 0.05$ ).

Regarding the difference between pancreatitis and healthy subjects, respectively healthy neoplasm, a sufficiently large area (AUC =  $0.49 \pm 0.11$  and AUC =  $0.656 \pm 0.09$ ) was not obtained and the statistical significance criteria were not met, p exceeding 0.05 in both cases ( $p = 0.93$  and  $p = 0.13$ ).

According to the ROC curve, for our group of patients, miR-10b would not have diagnostic significance (contrary to the literature), due to the low values of the area under the curve, but at the same time  $p > 0.05$ . The reason is again represented by the small and uneven number of patients enrolled in the study.

#### Sensitivity and specificity of markers

Sensitivity is the extent to which true positives are not missed/overlooked (so false negatives are few) and specificity is the extent to which positives really represent the condition of interest and not some other condition being mistaken for it (so false positives are few).<sup>22</sup> If we want our marker to have a high sensitivity, we lower the threshold value and we will not get rid of any pancreatic cancer, but we will diagnose as neoplastic the patients who do not have the disease – in other words, lower the specificity; on the other hand, if we want a high specificity, we increase the threshold value and then we will be more certain that a patient with the positive test has the disease, but we will have many false negatives, so we will get rid of patients with pancreatic cancer, we diagnosed – in other words, lowering the sensitivity of the test. So, for a given test, we can increase sensitivity with the cost of declining specificity and vice versa.

For the comparison of neoplasm versus pancreatitis, the threshold value with the best balance between the sensitivity and specificity of mesothelin is 6076 ng/mL, being the closest to the upper left corner of the graph – Sn = 0.97, Sp = 0.93; for mesothelin value = 5656 ng/mL, Sn = 0.99, Sp = 0.68; for mesothelin = 7796 ng/mL, Sn = 0.91, Sp = 0.65.

The threshold value of mesothelin for differentiation between neoplasm and healthy is 6636 ng/mL (Sn = 94%, Sp = 90%), having a mesothelin value of 6436 ng/mL for a 100% specificity or a value of 6696 ng/mL for a 95% sensitivity, while for pancreatitis vs control the threshold value of mesothelin is 6996 ng/mL with sp = sn = 80%.

#### Correlation between markers

The Pearson Coefficient is a statistical technique that measures and describes the degree of linear



association between two normally distributed quantitative variables.

Thus, the correlation coefficient obtained between mesothelin and miR-10b was  $r = -0.08$ . Its negative value indicates an inverse relationship between the two markers, but  $r [0; 0.2]$ , which shows a very poor correlation, is statistically insignificant ( $p = 0.53$ ).

Due to the fact that many of the patients studied did not have TNM staging, most of them being hospitalized for stenting and not for surgical treatment, we have chosen to classify the severity of the case according to tumor size. Following the calculation of the Spearman correlation index, there was a direct correlation between tumor size and miR-10b, but no correlation between mesothelin and tumor diameter.

**The strengths of this study** include its comprehensive search strategy using a world-wide medicine platform for research, without language restriction; the interdisciplinary character of the project with the collaboration between gastroenterology physicians for clinical and imaging assessment of patients, laboratory physicians, immunologists, biological scientists for genetic and serological determinations.

Of further concern are the **study limitations**:

- prospective study at the outset, enrolling patients only within one year;
- the selection of patients in the neoplasm group did not take into account the type of cancer, localization or staging;
- groups of patients not very well balanced in terms of number, distribution by gender and age;
- study in an emergency hospital, with a limited number of chronic illnesses;
- not all patients had staging of pancreatic cancer and so could not explain the high variations in marker concentrations;
- the small number of markers tested;
- mesothelin may have elevated levels in ovarian adenocarcinomas, squamous cell carcinomas, lung neoplasms or mesotheliomas;
- the use of two different methods of determining marker concentration (ELISA and qPCR)

We plan to control these factors by creating a bigger and more balanced lot of patients. We will exclude from the beginning those who are not adherent to treatment and do not come back for regular check-ups. Also, we will try to establish a collaboration with the general surgery department for tissue samples during pancreatic cancer surgery and maybe with some sponsorship we will be able to perform the missing tracking tests and markers.

## CONCLUSIONS

The main issue in the management of the pancreatic cancer is the lack of a set of biomarkers for an early diagnosis. This is extremely important, knowing that survival and prognosis depend on the tumor stage at the moment of diagnosis. The early diagnosis accompanied by small size tumor resection is usually associated to the best prognosis.

According to the numerous studies on this topic, we may state that by the large implication in the cellular mechanisms for regulation of the cellular cycle, in the DNA repair, the control of apoptosis and in the mechanisms of cancer spreading, miRNAs may be used as potential biomarkers for the clinical management of the pancreatic cancer.

Due to increased incidence and high mortality, including in detectable cases of neoplasm, the discovery and establishment of a marker or a panel of markers for early screening and early diagnosis of pancreatic cancer should be a priority in the research undertaken in this field.

## Compliance with Ethics Requirements:

*„The authors declare no conflict of interest regarding this article“*

*„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“*

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