DIPLOMARBEIT

Elevated amylase in plasma represents an adverse prognostic marker in patients with metastatic pancreatic cancer

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Disclosure

This thesis was the basis for the elaboration of a manuscript, which has been published in Wiener Klinische Wochenschrift. The published manuscript " Elevated amylase in plasma represents an adverse prognostic marker in patients with metastatic pancreatic cancer"(1) was drafted by the doctoral candidate, Eva Asamer. Therefore, significant parts of the thesis are similar to the published manuscript (with permission of Wiener Klinische Wochenschrift).

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Zusammenfassung

Das Pankreaskarzinom stellt unter allen Krebserkrankungen jene mit der schlechtesten Prognose dar. Trotz intensiver Forschung gelang es in den letzten Jahrzehnten nicht, die Lebenserwartung signifikant zu verlängern. Viele verschiedene Medikamente und Therapieschemata wurden getestet und geprüft, dennoch ist und bleibt die totale Resektion in frühen Stadien die einzige kurative Therapieoption. Unglücklicherweise wird der Großteil der Patientinnen und Patienten erst im fortgeschrittenen Krankheitsstadium vorstellig, sodass letztendlich eine palliative Chemotherapie und weiter "Best Supportive Care" die Therapie der Wahl darstellt.

Um das individuelle Outcome der Patienten vorherzusagen sind nur wenige prädiktive Parameter wie zum Beispiel der Tumormarker CA19-9 oder spezifische Parameter der systemischen Immunantwort von Bedeutung. Nun stellt sich die Frage, ob die pankreasspezifischen Laborparameter Amylase und Lipase nicht auch als ein prädiktiver Wert für die individuelle Prognose von Pankreaskarzinompatienten verwendet werden könnten. In dieser Arbeit wurde versucht, diese Frage zu beantworten, mit dem Ergebnis, dass Amylase tatsächlich mit dem Überleben der Patientinnen und Patienten korreliert - je höher der Laborwert zum Zeitpunkt der Diagnose, desto schlechter das klinische Outcome der Patientin bzw. des Patienten.

Auch wenn der Lipase- und Amylase- wert zwar miteinander korrelieren, gilt dies jedoch nicht gleichermaßen für den Lipasewert. Dieser hat laut diesen Berechnungen keine Aussagekraft bezüglich der individuellen Prognose bei Pankreaskarzinompatienten. Da der Amylasewert einen leicht und nicht invasiv zu erhebenden Parameter darstellt, wäre er eine praxistaugliche, geschickte und kostengünstige Hilfestellung um Pankreaskarzinompatienten in verschiedene Risikogruppen zu stratifizieren.

Abstract

Background and aim: Clinical outcome of metastatic pancreatic cancer (PC) patients is dismal and novel prognostic factors might help to stratify patients into different risk groups in clinical trials and clinical decision-making. Lipase and Amylase are recommended markers for diagnosis of acute pancreatitis, but their significance with regard to prognostic relevance in metastatic PC is uncertain. The aim of this study was to investigate the prognostic relevance of these two parameters concerning survival of patients suffering from metastatic PC.

Method: This large retrospective study includes 351 patients with metastatic PC, who were treated between the years 2004 and 2015 in a single academic institution. Cancer-specific survival (CSS) was analysed using the Kaplan-Meier method. To further evaluate the prognostic significance of lipase and amylase, univariate and multivariate values were calculated using Cox proportional models.

Results: In our study cohort, amylase and lipase values were highly correlated (R=0.821, p<0.001). In univariate analysis, an increased amylase level was associated with shorter CSS in PC patients (HR=1,258; 95%CI=1,011-1,566; *p*=0,039). In multivariate analysis, including gender, age, CA19-9 and administration of chemotherapy, increased amylase levels prevailed as an independent prognostic factor for CSS (HR=1,373; 95%CI=1,004 – 1,878; *p*=0.047).

Discussion: Plasma amylase was identified as an independent prognostic factor in metastatic PC patients. Our results indicate that amylase might represent a novel and useful marker for better patient stratification in PC management.

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1 Introduction

Pancreatic cancer (PC) is currently associated with the lowest survival rates among all known malignancies, a fact that has hardly changed over the last decades. With over 55.440 new cases each year and 44.330 PC related deaths, the demand for a better understanding of this lethal disease becomes all the more evident.(2) During the last decade, many studies have been conducted in order to improve the overall survival rate of PC; yet, the only potential chance for a cure remains to be total resection.(3) However, merely 15 to 20 percent of patients can be considered for surgical resection and pancreatectomy, respectively, as surgery can only succeed, if the tumor is still in its early stages and has not formed distant metastases.(4) Unfortunately, at the time of diagnosis, the majority of patients present with either locally advanced or metastatic disease.(5,6) To predict the duration of patients' clinical outcome, only a small variety of parameters, apart from radiological examinations, are frequently employed. Numerous studies, investigating the tumor marker CA19-9, have shown that there is a correlation between the level of CA19-9 and the survival rate of patients with advanced disease.(7,8) Other laboratory markers including the systemic inflammatory response have been extensively studied and were proposed as useful in prognosis of PC patients.(9–12) The question posed in this study was, whether the pancreatic enzymes lipase and amylase in plasma can be used as prognostic predictors as well. This is especially significant, as determining a blood-based marker is a non-invasive and gentle method towards cancer patients. The enzymes addressed in this study are commonly tested for diagnostic assessment on suspicion of acute pancreatitis, but their significance with regard to cancer patients has hardly been considered.(13)

Currently, there is no data in PC regarding amylase and lipase as prognostic markers. Therefore, the aim of the present study is to explore, whether measured amylase and lipase levels can lead to a statistically significant improvement of prognostic prediction.(1)

1.1 Epidemiology and Risk Factors

In the ranking of worldwide death causes elaborated by the World´s health organisation "GLOBOCAN Database", pancreatic cancer in both men and women is currently placed on rank seven. However, there are significant differences of incidence depending on the geographic location:

> *"The age-standardized rate (ASR) incidence was highest in Europe (7.7 per 100,000 people) and North America (7.6 per 100,000 people), followed by Oceania (6.4 per 100,000 people). The lowest rate was observed in Africa with an estimated incidence of 2.2 per 100,000 people [1]. Differences in incidence rates were 30-fold between the populations at the highest rate (Hungary: 10.8), and the populations with the lowest rate (Guinea: 0.35)" (*See*:Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors"*Rawla, Sunkara, et.al., World J Oncol. 2019 Feb, 10-27)

The cause for these distinctions is not yet completely understood. Nevertheless, environmental circumstances, such as developed or undeveloped geographic areas, as well as certain risk factors, such as smoking, obesity or region-specific preferences with regard to nutrition might be considered as factors that correlate to the differences in incidence.(14) In addition to this, the disparate availability of diagnostic modalities and the differences in registration qualities assigned to pancreatic cancer patients in the different countries must also be taken into consideration.(15)

1.1.1 Risk Factors:

Apart from the epidemiologic factors mentioned above, there are still other aspects that feature prominently in the assessment of pancreatic cancer risk. When looking at such risk factors, they are generally divided into two different groups: modifiable and non-modifiable risk factors.(16)

1.1.1.1 Non -modifiable risk factors

First of all, the non-modifiable risk factors are comprised of gender and age. They further include ethnicity, genetic factors and family history, or previously diagnosed indications, such as chronic infections of the pancreas or diabetes mellitus.(17) Other causing factors

are for example the degree to which the body is able to detoxify tobacco products, the existence of oncogene mutations or biomarker immune expression.(18,19)

To go into more detail, each ethnicity has its own individual genetic and molecular build-up, which can affect the risk of developing PC either in an adverse or a positive way. Studies have shown that the expressions of K-RAS and p53 in Asian PC patients differ from Caucasian PC patients.(20) Therefore, according to Longnecker et al., it could be assumed, that Asian PC patients might have a better overall survival rate than patients from other ethnicities.(21)

If a first degree relative (parent, sibling or child) has also been diagnosed with pancreatic cancer, it poses another leading risk factor for the development of PC. The risk of individuals whose first grade relatives have been diagnosed with PC, have a nine-fold higher risk over the general population.(22) If at least two such relatives were diagnosed with PC this risk even doubles.(23) About 5 to 10 percent of all PC patients have a positive family history.(24,25)

Studies have shown that there are many different germline -mutations that might lead to an increased risk for developing PC. In about 10 percent of all PC cases certain genetic predispositions or germline -mutations were involved.(26) BRCA 2, for instance, seems to be one of the most important mutations, as it could be found in 17 percent of families with familial PC.(27) Numerous other germline mutations have been identified to favor the development of PC such as BRCA1, PALB2, ATM, CDKN2, APC, MLH1, MSH2, MSH6, PMS2, PRSS1 or STK11.(26,28) Apart from that, there exist several syndromes which increase the risk, such as the Lynch Syndrome, Peutz-Jaeghers Syndrome or the Li-Fraumeni Syndrome.(17)

However, there are several somatic mutations as well, such as the inactivation of the tumor suppressor genes: p53, p16/CDKN2a and SMAD4, stimulating the oncogene KRAS or inhibiting the genome maintenance genes MLH1 and MSH 2.(29)

Furthermore, both Diabetes mellitus I and II are associated with a higher risk for PC. Diabetes seems to be a leading cause for up to 9.7 percent of PCs.(30,31)

Some studies show that infections with helicobacter pylorii or hepatitis B and C seem to pose a risk for the development of the disease as well.(32,33)

1.1.1.2 Modifiable Risk factors

On the other hand, there are modifiable risk factors, which are thought to interact with non-modifiable risk factors, yet increase the risk of developing PC on their own as well.(17) Modifiable factors include smoking, obesity, consumption of alcohol or dietary differences with smoking representing the most prominent factor.(34) The risk appears to double in patients who smoke versus patients who do not smoke. Studies also show that the risk in current smokers is much higher than in former smokers. Furthermore, for ten years after smoking cessation, the risk seems to remain stable.(35,36) Passive smoking also increases the risk of PC for up to 50 percent.(35,37)

Next to smoking, obesity represents another strong risk factor for PC.(38) Studies have shown that there is an association between young adulthood obesity (BMI = 25 -<29 or BMI = >30) and the incidence of PC. However, the risk does not vanish considering obesity at an older age (BMI = 25 -<29 or BMI = >30; age= 30-79 years) but represents an adverse prognostic marker for diseased PC patients.(39) Considering the kind of fat and its respective correlation to PC as yet another risk factor it can be seen that both general and abdominal fat mass heighten the risk for PC.(40)

Having a look at different dietary types, red meat, processed meats, fried food or other foods which contain nitrosamines may have an adverse impact on the development of PC.(41,42)

The intake of strong alcohol, such as liquor and spirits, poses a further important risk factor for PC. However, there appears to be no association between the intake of wine or beer and the development of PC. Besides, the risk strongly depends on the amount of alcohol intake per day: for medium or low alcohol abuse the risk does not seem to increase significantly.(43–45)

1.2 Mortality rates

The lifetime probability of being diagnosed with an invasive disease in general but also with pancreatic cancer is slightly higher for men (39.7%) than for women (37.6%).(46) The cause of this inequality is still unclear, however environmental exposure, endogenous hormones and adult height seem to play a central role when it comes to different cancer development between men and women.(47)

With 55.440 new cases and 44.330 death cases each year, PC denotes a survival rate of 8 %(46) *(Figure 1,2,3)*

Estimated New Cases

			Males	Females			
Prostate	164,690	19%			Breast	266,120	30%
Lung & bronchus	121,680	14%			Lung & bronchus	112,350	13%
Colon & rectum	75,610	9%			Colon & rectum	64,640	7%
Urinary bladder	62,380	7%			Uterine corpus	63,230	7%
Melanoma of the skin	55,150	6%			Thyroid	40,900	5%
Kidney & renal pelvis	42,680	5%			Melanoma of the skin	36,120	4%
Non-Hodgkin lymphoma	41,730	5%			Non-Hodgkin lymphoma	32,950	4%
Oral cavity & pharynx	37,160	4%			Pancreas	26,240	3%
Leukemia	35,030	4%			Leukemia	25,270	3%
Liver & intrahepatic bile duct	30,610	4%			Kidney & renal pelvis	22,660	3%
All Sites	**856,370**	**100%**			**All Sites**	**878,980**	**100%**

Estimated Deaths

			Males	Females			
Lung & bronchus	83,550	26%			Lung & bronchus	70,500	25%
Prostate	29,430	9%			Breast	40,920	14%
Colon & rectum	27,390	8%			Colon & rectum	23,240	8%
Pancreas	23,020	7%			Pancreas	21,310	7%
Liver & intrahepatic bile duct	20,540	6%			Ovary	14,070	5%
Leukemia	14,270	4%			Uterine corpus	11,350	4%
Esophagus	12,850	4%			Leukemia	10,100	4%
Urinary bladder	12,520	4%			Liver & intrahepatic bile duct	9,660	3%
Non-Hodgkin lymphoma	11,510	4%			Non-Hodgkin lymphoma	8,400	3%
Kidney & renal pelvis	10,010	3%			Brain & other nervous system	7,340	3%
All Sites	**323,630**	**100%**			**All Sites**	**286,010**	**100%**

Figure 1: Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2018.

Ranking is based on modeled projections and may differ from the most recent observed data.
Adapted from: "Cancer Statistics, 2018" by Siegel RL, Miller KD, Jemal A, et.al., published in 2018 in *A Cancer Journal for Clinicans,* volume 68, pages 7-30

Different to the majority of invasive cancer diseases, the survival of black skinned PC patients is higher than for white skinned people who suffer from PC.

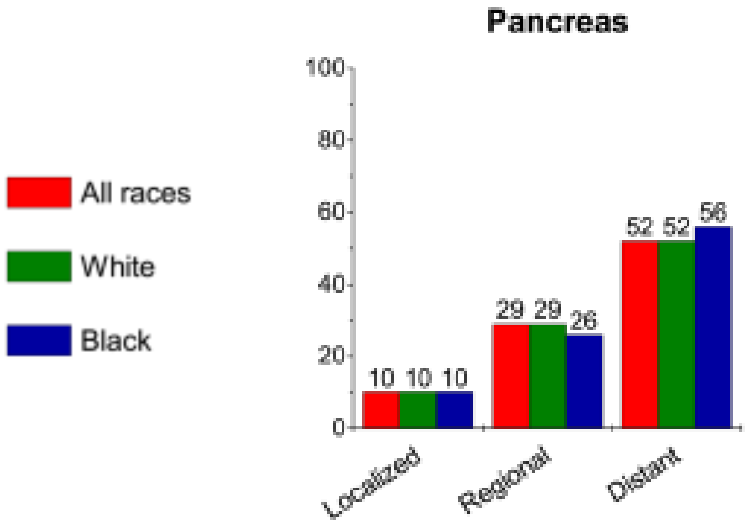

Figure 2:Five-Year Relative Survival Rates for Selected Cancers by Race and Stage at Diagnosis, United States, 2007 to 2013.

Adapted from: "Cancer Statistics, 2018" by Siegel RL, Miller KD, Jemal A, et.al., published in 2018 in *A Cancer Journal for Clinicans,* volume 68, pages 7-30

1.2.1 Trends:

A mortality trend is probably the most important indicator of progress against cancer as it is not influenced by new detection tools but only by incidence and survival.(48) Due to the consumption of tobacco, the overall cancer survival rate increased during the 20th century but started dropping for 1,5% per year again since 1990.(46) Whereas the death rate for lung-, breast-, colorectum- or prostate cancer dropped significantly over the last 2 decades, the mortality trend for pancreatic cancer remained stable.

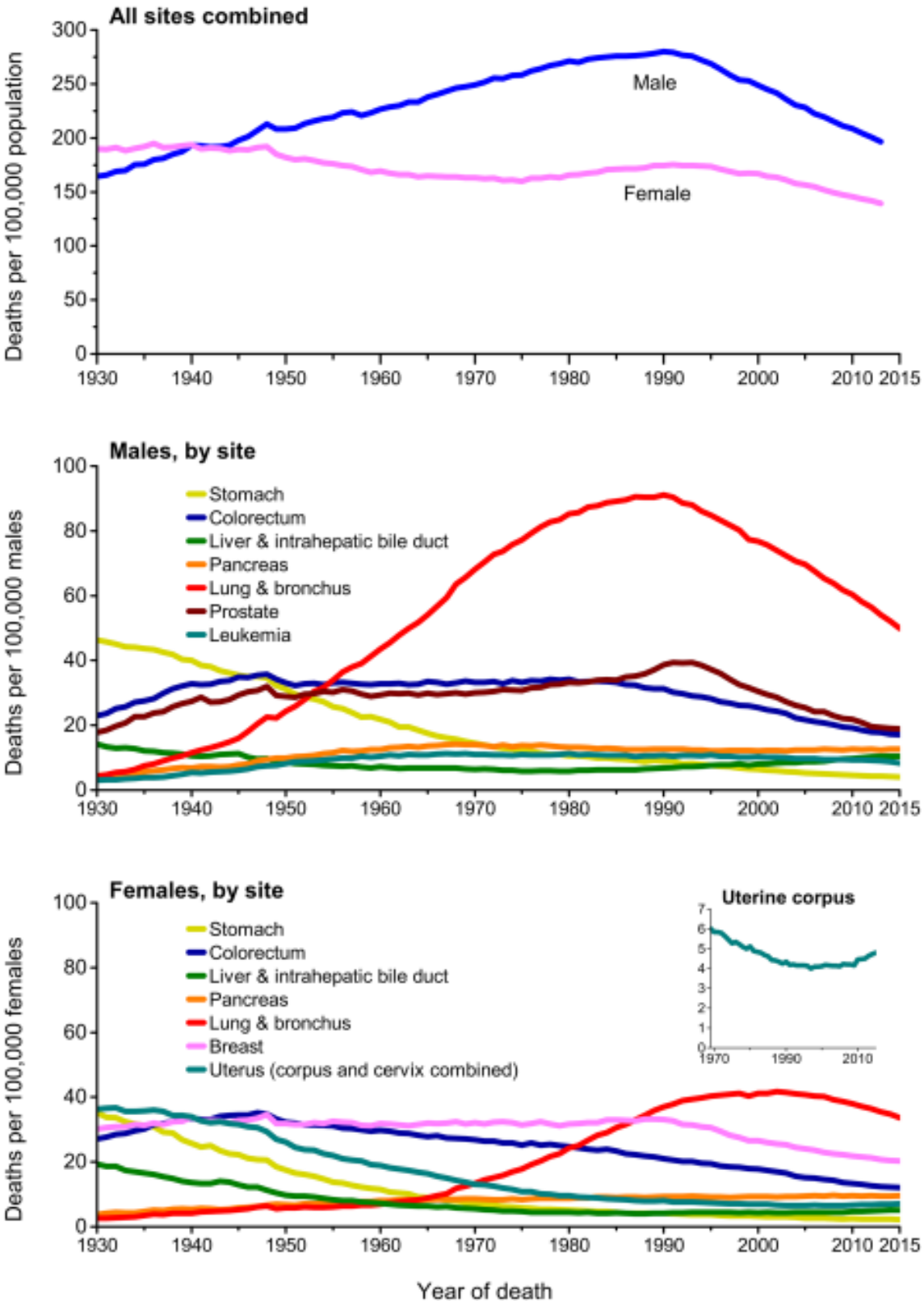

Figure 3:Trends in Cancer Death Rates by Sex Overall and for Selected Cancers, United States, 1930 to 2015.

Adapted from: "Cancer Statistics, 2018" by Siegel RL, Miller KD, Jemal A, et.al., published in 2018 in *A Cancer Journal for Clinicans,* volume 68, pages 7-3

1.3 Clinical Manifestation

The most frequent symptoms of patients with PC are not particularly different from those generally associated with malignancies of other origins. Clinical Manifestations, such as weight loss, asthenia or anorexia - the typical B-symptoms, occur in over 80 percent of PC cases.(49) However, PC may present with various more specific symptoms, such as jaundice, dark urine, epigastric pain or steatorrhea as well. Which one of the various specific symptoms appearing in PC patients makes itself felt, depends on the location of the tumor.(50) As mentioned above, the majority of pancreatic malignancies are located in the head of the pancreas, whereas only 20 to 25 % grow in the body and the tail.(50) If the tumor is located in the head of the pancreas, jaundice and steatorrhea are the symptoms to be expected, because of the potential occlusion of the common bile duct and the main pancreatic duct, respectively. The occlusion of the main pancreatic duct leads to a blockage of digestive enzymes, such as lipase, which is responsible for the digestion of fat, and, therefore to steatorrhea eventually. Jaundice, if truly caused by the occlusion of the common bile duct, causes hyperbilirubinemia of the cholestatic type (increase of the conjugated fraction of bilirubin) and as a consequence pruritus, dark urine and white stool.(49,51)

1.3.1 Pain

Another clinical manifestation that should not be disregarded in this work is pain, which is undoubtedly one of the most common and oftentimes reported symptoms in patients with PC.(52) When measuring the strength of the pain, the size of the tumor seems not to be of prime importance, as even tumors of a size below 2 cm may cause the typical gnawing visceral pain, which usually starts epigastric and may spread to the sides like a belt.(53,54) Moreover, progressively developing back pain without any orthopedic correlation should ring alarm bells as it could be a symptom for a tumor of primarily the body but also of the tail of the pancreas. This can also be observed in tumors of the head of the pancreas, yet malignancies growing in the body or the tail of the pancreas are more likely to show perineural invasion, which is responsible for the severe back pain in the majority of cases.(55)

It is reported that this particular kind of pain often gets worse during the night, while eating or when lying supine.(54)

1.3.2 Other symptoms

There are numerous other clinical manifestations of PC, which may be less frequent than the ones mentioned above but are still important to be kept in mind.

Malignancies of the pancreas may induce, for example, an emerging diabetes mellitus. If diabetes occurred for the first time in the last two years, it is rated as “recent onset of diabetes mellitus” which PC is responsible for in 25% of cases.(56)

In rare cases unexplained superficial thrombophlebitis or other skin manifestations are reported as clinical manifestations of PC.(57,58)

As in many cases PC has already spread to other organs at the time of diagnosis, PC patients often show signs of metastatic disease as well. PC usually spreads haematogenously, as well as lymphogenic. It frequently affects liver, perineum, lungs and in some cases also bones. Ascites, abdominal mass, left supraclavicular lymphadenopathy or a palpable periumbilical mass are reported as signs of metastatic pancreatic cancer.(50,59)

1.4 Staging

To ensure appropriate therapeutic progress for PC patients, an early diagnosis and accurate staging are decisive and compulsory. Once a pancreatic malignancy is suspected in initial imaging, the next step is usually the staging. Tumor staging aims to stratify patients into different therapy groups in order to minimize surgical or therapeutic morbidity and mortality.(60) Numerous imaging techniques are in clinical use for tumor staging, for example: computed tomography (CT), Magnetic resonance imaging, positron emission tomography (PET) with FDG as tracer, endoscopic ultrasound, endoscopic retrograde cholangiopancreatography (ERCP) or diagnostic laparoscopy. The main role of these imaging devices is to identify a lesion and to detect its malignant potential. Moreover, it is important to determine the resectability of the lesion to avoid unnecessary or impossible surgeries and to identify the most suitable therapy for the patient as soon as possible. To stage pancreatic tumors, especially the adenocarcinoma, multidetector CT or PET with a sensitivity and specificity for the diagnosis of pancreatic cancer of 88.5% and 70.6%, respectively, are today´s tools of choice.(60,61) The common way to stage a PC patient into groups from Ia – III/ IV follows the tumor - node - metastasis - (TNM) system of the combined American Joint Committee on Cancer/ Union for International Cancer Control which is being renewed on a regular basis. The current version is depicted in *Table 1*.(62,63) Survival rates for different staging groups are provided in *Figure 4* below.

Table 1: Exocrine pancreatic cancer TNM staging AJCC UICC 2017

Primary tumor (T)			
T category	T criteria		
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ.		
T1	Tumor ≤2 cm in greatest dimension		
T1a	Tumor ≤0.5 cm in greatest dimension		
T1b	Tumor >0.5 cm and <1 cm in greatest dimension		
T1c	Tumor 1 to 2 cm in greatest dimension		
T2	Tumor >2 cm and ≤4 cm in greatest dimension		
T3	Tumor >4 cm in greatest dimension		
T4	Tumor involves celiac axis, superior mesenteric artery, and/or common hepatic artery, regardless of size		
Regional lymph nodes (N)			
N category	N criteria		
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastases		
N1	Metastasis in one to three regional lymph nodes		
N2	Metastasis in four or more regional lymph nodes		
Distant metastasis (M)			
M category	M criteria		
M0	No distant metastasis		
M1	Distant metastasis		
T1	N0	M0	IA
T1	N1	M0	IIB
T1	N2	M0	III
T2	N0	M0	IB
T2	N1	M0	IIB
T2	N2	M0	III
T3	N0	M0	IIA
T3	N1	M0	IIB
T3	N2	M0	III
T4	Any N	M0	III
Any T	Any N	M1	IV

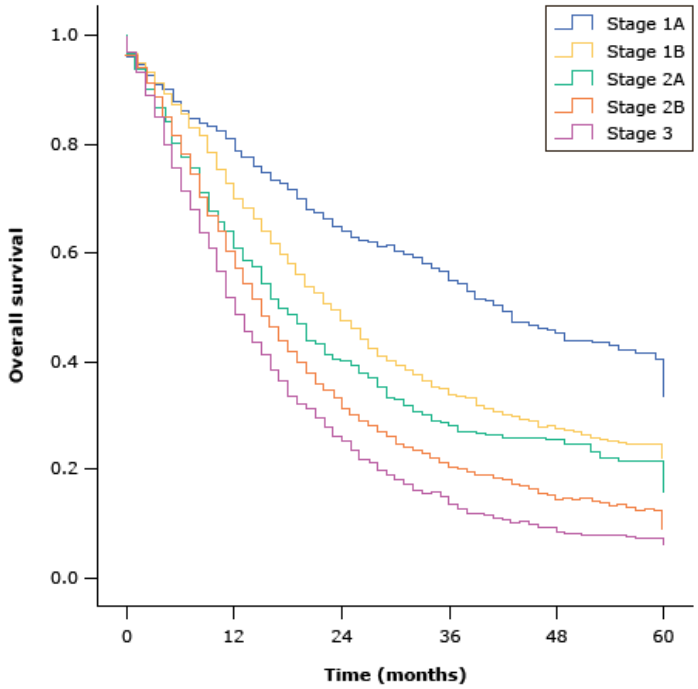

Figure 4: Predicted overall survival for patients with resected pancreatic cancer according to the 8th edition (2017) of AJCC: prognostic stage groups.

Adapted from: "Annals of Surgical Oncology, Validation of the American Joint Committee on Cancer, 8th Edition Staging System for Patients with pancreatic adenocarcinoma: A Surveillance, Epidemiology and End Results (SEER) Analysis" by Kamarajah SK, Burns WR, Frankl TL, et al, published in 2017 in *Society of surgical oncology*, vol. 24, 2017, p. 2023

1.4.1 CT Technique for PC Staging

As mentioned above, the multidetector CT is a commonly used device for PC staging. The preferred technique is the triple- phase contrast- enhanced thin sliced (multidetector – row) helical computed tomography with three dimensional reconstruction. This method enables the examiner to detect smaller tumors and to evaluate the main pancreatic duct more precisely.(64,65)

For extensive examination of a suspected malignancy of the pancreas the patient should be scanned in three dynamic phases of contrast injection ("pancreas protocol").(66) The first phase, called the arterial phase of enhancement, permits excellent examination of the celiac axis, the superior mesenteric artery and the peripancreatic arteries.(66) The "pancreatic phase" happens between the peak of the arterial phase (aorta) and peak enhancement of the liver, which occurs in the portal venous phase.(67,68)

Last but not least, to detect hepatic metastasis and to have a better view on the superior mesenteric vein, splenic and portal veins, the portal phase, which hits after 60 to 70 seconds after contrast injection becomes involved.(69)

Based on this triple – phase staging method an initial assessment of resectability can be made which is mostly dependent on the involvement of adjacent structures. A verdict of unresectability is in the majority of cases reached, due to local vascular invasion.(70)

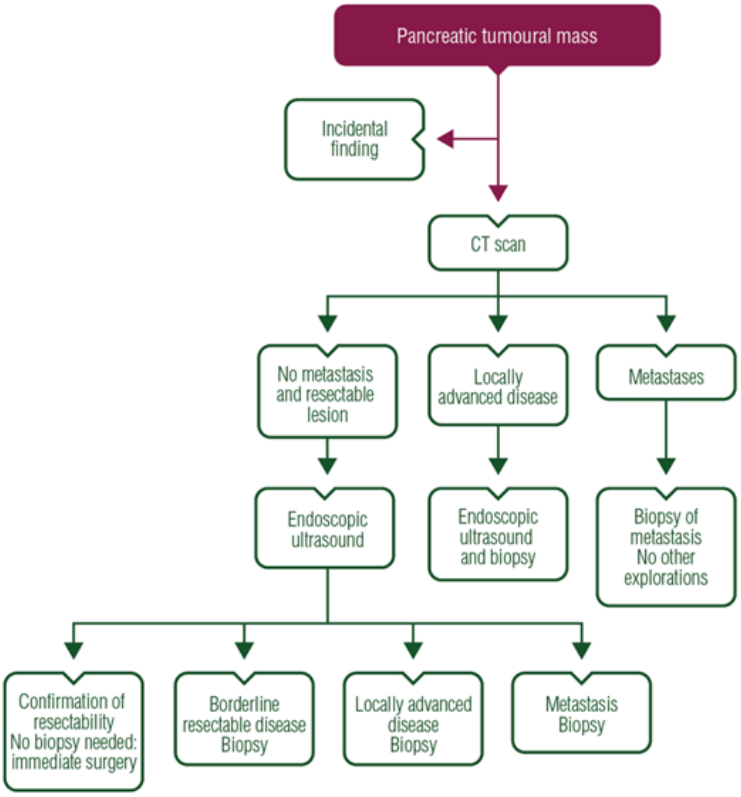

Figure 5: Diagnsotic work-up before multidisciplinary decision. CT, computed tomography

Adopted by: "Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up" by Ducreux M., Cuhna A. Sa., et.al., published in 08/2015 in *Annals of Oncology,* volume 26, pages v56-v68

1.5 Histologiocal and Immunohistochemical types and subtypes of malignant pancreatic lesions

In order to identify the true histopathological subtype of a pancreatic tumor, a surgical resection or at least a biopsy – either fine needle aspiration (FNA) or a core needle biopsy - of the suspicious lesion becomes necessary. Especially further ways of treatment strongly depend on the result that can be gleaned from examining the collected tissues.(71)

1.5.1 Ductal adenocarcinoma

With 85%-90%, the ductal adenocarcinoma represents the most frequent of all malignant tumors of the pancreas. The incidence peak occurs at the age of 66 years, slightly more often in men. The ductal adenocarcinoma grows as a ductal and solid tumor. The mass is normally rigid and of white or yellow color. The main pancreatic duct may be extended and the remaining pancreatic tissue is often atrophic.(72)

If examined under the microscope it has glandular and ductal structures. The cells with enlarged pleomorphic nuclei and eosinophilic to clear cytoplasm are commonly surrounded by abundant and desmoplastic stroma. The tumors often show perineural, lymphatic and blood vessel invasion.(72)

Immunohistochemically observed, the ductal adenocarcinoma can be noticed by an abnormal TP53 expression, SMAD4 loss and expression of MUC1, MUC3, MUC4, MUC5AC and, last but not least, the expression of CA19-9, which represents one of the most important tumor markers.(72) A study by Maitra A., Hruban RH., et.al., has also pointed out a remarkably frequent activating point mutation of KRAS2 (in over 90%), as well as inhibiting mutations in the p16/CDKN2A (in over 90%) and MADH4/DPC4 (55%) genes.(73) The evidence of the loss of the MADH4/DPC4 expression strongly suggests the existence of a ductal adenocarcinoma, which can be helpful considering the origin of carcinomas of unknown primary.(26) *(Figure 6)*

However, if this specific mutation can be found, it has an adverse effect on the patients´ overall survival.(74)

The ductal adenocarcinoma shows usually 20 up to 80 different somatic mutations.(72) The germline mutations mentioned in the chapter “non-modifiable risk factors” must of course be kept in mind as well.

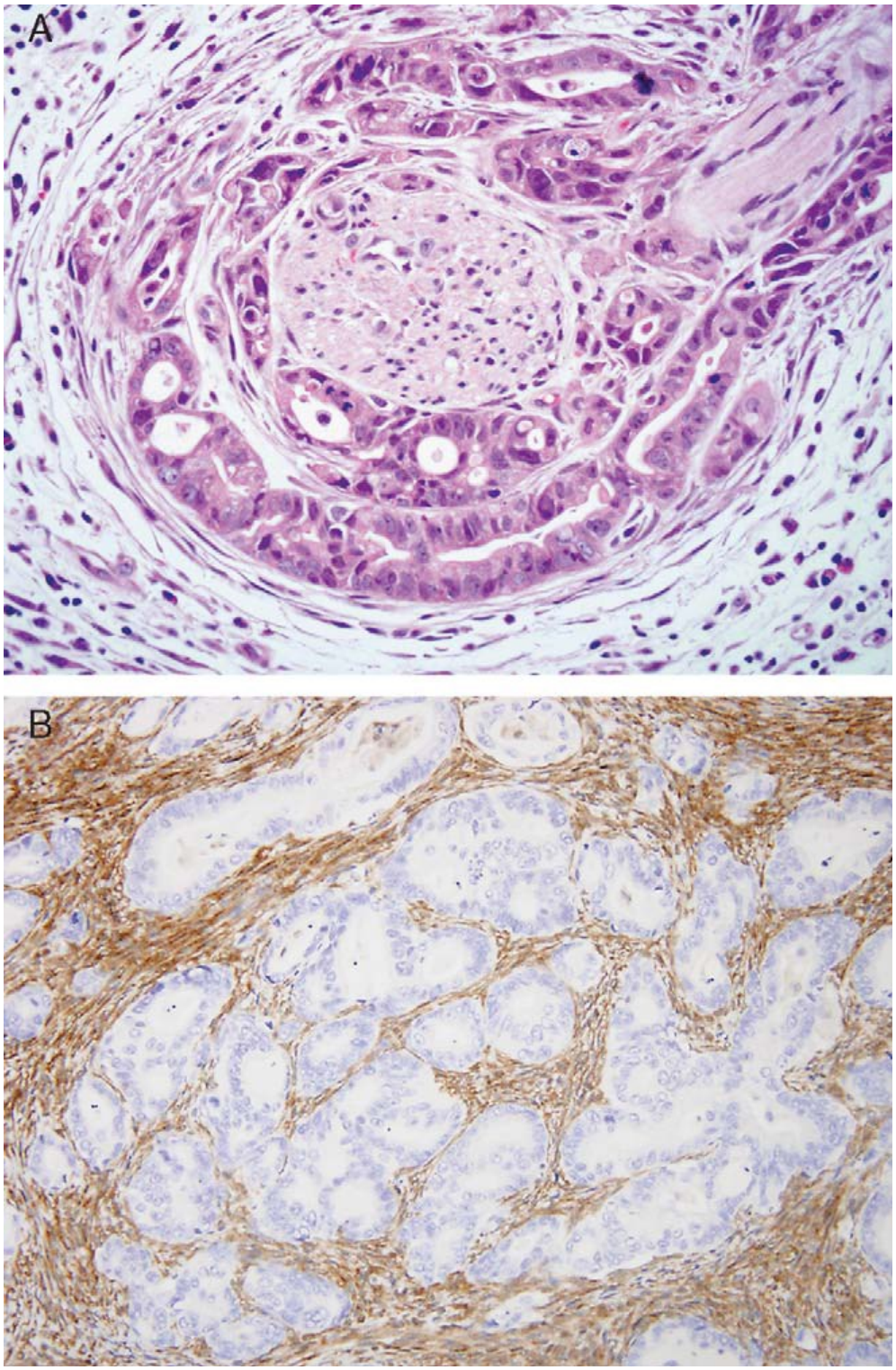

Figure 6: Infiltrating ductal adenocarcinoma.

A, Neoplastic cells are seen surrounding a nerve.
B, Pancreatic ductal adenocarcinoma metastatic to the ovary. Neoplastic cells show loss of immunohistochemical labeling for dpc4 protein,supporting a pancreatic origin.
Adapted from: "Molecular characterization of pancreatic neoplasms" by Shi C., Daniels J., et. Al., published in 07/2008 in *Advances in anatomic pathology*, volume 15(4), pages 185-195
License number: 4602970540708

1.5.1.1 Precursor lesions

It is well known that the ductal adenocarcinoma of the pancreas develops with precursor lesions. The pancreatic intraductal neoplasia (PanIN) is the origin of the majority of ductal adenocarcinomas of the pancreas and it usually occurs in microscopic ducts with a size of less than 5 mm.(75,76) It can, therefore, not be detected by using imaging techniques. The precursor lesions: intraductal papillary mucinous neoplasms (IPMN) or mucinous cystic neoplasms (MCN), on the other hand, can sometimes be coincidentally depicted on MR/CT images.(76)

PanIN lesions can be distinguished into three groups: PanIN-1A,PanIN-1B, PanIN-2 and PanIN-3. PanIN-1A and PanIN-1B count as low grade dysplasia, PanIN-2 as intermediate grade dysplasia and PanIN-3 is considered as carcinoma-in.situ or high grade dysplasia.(76) (figure 6)

A study from Konstantinidi IT, Vinuela EF, et.al., has shown that in 153 out of 584 patients, who underwent pancreatectomy caused by something other than a ductal adenocarcinoma of the pancreas, a PanIN lesion could be found.(77)

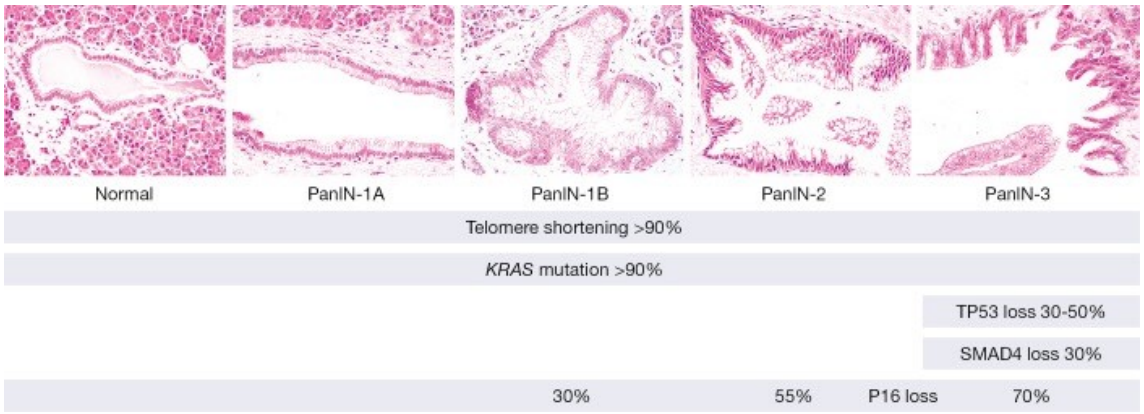

Figure 7: PanIN progression model of pancreatic cancer

Each step in the progression from normal epithelium to low-grade PanIN, and on to high-grade PanIN is accompanied by accumulating genetic alterations. From left to right: a normal pancreatic duct is lined by cuboidal to low-columnar epithelium with amphophilic cytoplasm.
PanIN-1A shows flat epithelial lining with tall columnar cells with basally located nuclei and abundant supranuclear mucin.
PanIN-1B identical to PanIN-1A except for a papillary, micropapillary, or basally pseudostratified architecture in PanIN-1B.
PanIN-2 demonstrates full-thickness pseudostratification of nuclei with mild-to-moderate cytologic abnormalities.
PanIN-3 is characterized by complete loss of polarity, budding of cellular tufts into the duct lumen, and significant nuclear pleomorphism. PanIN, pancreatic intraepithelial neoplasia.
Adopted with permission from AME publishing company by: „Pancreatic adenocarcinoma pathology:“changing landscape““by Brosens L., Hackeng W., et.al., published in 08/2015 in *Journal of gastrointestinal oncology,* volume 6(4), pages 358-374

1.5.1.2 Microenvironment and the effect of chemotherapeutics

Considering the microenvironment of ductal adenocarcinomas of the pancreas, as well the surrounding stroma and its different expressions seem to have a significant influence on the overall survival rate. Studies have shown that these tumors might be able to develop their own immunosuppressive surrounding with expressing regulatory T-cells. Therefore, it becomes more difficult for nowadays widespread immune based therapies to detect and destroy the shielded tumor cells.(78)

Additionally, the overexpression of hyaluronic acid and collagens might play an important role for the effect of a chemotherapy, as the surplus of hyaluronic acid and collagens leads to an increased interstitial fluid pressure and consequently to a worse passive transport of chemotherapeutics.(78)

1.5.2 Pancreatic neuroendocrine tumor/carcinoma

The neuroendocrine tumors of the pancreas are with 5% prevalence on rank 2 of all malignant pancreatic lesions with the mean age at 58 years.(79,80) Just as the ductal adenocarcinoma, also the neuroendocrine tumor occurs more often in men than in women (3:2). The differentiation is of endocrine origin. The macroscopic appearance of these tumors is commonly solid with a soft consistency, in some cases with cystic degeneration. The color is often red or white and the tumors are usually well circumscribed. Viewed microscopically, the cells neuroendocrine tumors of the pancreas show the typical “salt and pepper chromatin” in their nuclei surrounded by granular amphophilic to eosinophilic cytoplasm. The cells normally grow in nests or in trabecular constellations.(72)

The immunohistochemistry of neuroendocrine tumors of the pancreas shows characteristically an overexpression of synaptophysin and chromogranin, but also of the hormones insulin and glucagon. Concerning somatic mutations, the average number of mutations is 16, particularly an abnormal TP53 expression could be observed.(72) The overall survival of an neuroendocrine tumor of the pancreas depends on the subtype of the tumor. If the tumor expresses hormones it is called “functional” if the tumor does not express any hormones it is called “non-functional”. The 5.-year survival rate for functional tumors is 47,6% and 33.7% for non -functional tumors.(80)

1.5.3 Solid pseudopapillary neoplasms of the pancreas

The solid pseudopapillary neoplasm of the pancreas represents a rather uncommon type of pancreatic malignancy. Yet, with only 1-2% it is still the third most frequent malignant pancreatic lesion. Surprisingly, these tumors usually occur in young women with an age peak of 29 years.(81)

Macroscopically these tumors appear as solid, cystic degenerated, pseudopapillary masses with necrotic sections. Microscopically, pseudopapillary neoplasms show eosinophilic to clear cytoplasm with oval nuclei. The cells seem poorly cohesive with fine blood vessels running through.(72)

To tell the immunohistochemical difference between pseudopapillary neoplasms and other malignancies of the pancreas, it is important to point out that pseudopapillary tumors express CD10, abnormal labeling for paranuclear dot-like CD99 and β-catenin and a loss of membranous e-cadherin.(72)

Apart from that, pseudopapillary neoplasms stand out concerning the long term survival rate. After surgical resection the mortality rate appears to be less than 2% and the disease-free survival over 95%. These numbers strongly differ from those of the most common malignant neoplasms of the pancreas.(81)

1.5.4 Acinar cell carcinoma

Ranked fourth of all most common pancreatic malignancies, the acinar cell carcinoma occurs in less than 1% of all patients with malignant pancreatic tumors. The vast majority of these tumors occur in elderly people with a mean age of 65 years, more often in men than in women. However, 6% occur in patients aged 8 to 15 years, which is rather uncommon for pancreatic tumors, but might be due to an increasing number of acinar cell carcinoma in association with diverse hereditary syndromes.(72,82,83)

Acinar cell carcinomas are commonly solid than for example ductal adenocarcinomas of the pancreas. The tumor cells usually show granular eosinophilic cytoplasm and acinar units as their distinctive growth pattern.(79)

Acinar cell carcinomas frequently express typical pancreatic enzymes, such as trypsin, lipase or chymotrypsin, which may be detected in the patients´ blood in order to diagnose this particular histologic subtype of a pancreatic malignancy. Also, the expression of BCL10 could give a hint considering differential diagnosis.(72,84)

The 5 year survival rate strongly depends on whether the patient underwent surgical resection (72%) or not (42,8%).(85)

1.5.5 Pancreatoblastoma

Pancreatoblastoma is a rare malignant tumor with an age peak of 5 years. However, few cases of pancreatoblastomas presenting in adolescence or adulthood were reported. (86) To diagnose pancreatoblastomas histologically, an acinar differentiation but also squamoid nests must be existent. Generally, pancreatoblastomas might resemble any of the other pancreatic malignancies mentioned histologically and immunohistochemically which makes diagnostics more complicated.(72,86)
Pancreatoblastomas are associated with some hereditary syndromes but especially with the Beckwith-Widemann syndrome.(87)
The 5 year survival rate is around 50%.(86)

1.6 Therapy

The treatment of pancreatic cancer fully depends on the stage of the tumor. The disease may be categorized in resectable, locally advanced and metastatic cancer.(88,89). As this work pays particular attention to advanced pancreatic adenocarcinoma or metastatic adenocarcinoma, respectively, the treatment for this stage of disease is going to be discussed more precisely in the following paragraph.
Unfortunately, patients with metastatic pancreatic cancer usually do not receive a curative treatment, as total resection is no option anymore. The therapy is therefore limited to palliative measures and best supportive care.(89) Only smaller surgical interventions are still recommended, such as endoscopically placed metallic biliary stents in case of biliary and/or duodenal obstruction.(90) Apart from that, it is necessary to treat symptoms such as pain efficiently according to strict guidelines and to consult a specialist soon enough.(89)
In order to find a suitable treatment for patients with metastatic pancreatic cancer multidisciplinary collaboration is inevitable. Furthermore, and perhaps even more importantly, the patients individual expectations and wishes should be involved in the decision-making process.(91)

1.6.1 Chemotherapeutics and their properties:

The following chemotherapeutics may be considered for the treatment of advanced pancreatic cancer according to drug information:

Gemcitabine: Pyrimidin analogon

Initial dose of 1000mg/m^2 iv. in 30 minutes once a week for 7 weeks

one week rest

afterwards once weekly every three weeks out of 4 weeks

Nab-paclitaxel: Nanoparticle albumin-bound Paclitaxel: Taxane Derivative, Antimicrotubular

Premedication with Dexamethason, Diphenhydramine, ranitidine, Cimetidine, famotidine

Dose: 125 mg/m^2 iv. On day 1, 8 and 15 of a 28 day cycle

May be combined with Gemcitabine

CAVE: AST up to 10 times and Billirubin between 3 and 5 times higher

OR: AST 10 times higher and Billirubin over 5

No treatment is recommended!

FOLFIRINOX: Flourouracil, Leucovorin, Oxaliplatin, Irinotecan

Flourouracil: antineoplastic agent, Pyrimidine analogon

Leucovorin: chemotherapy modulating agent, antidote

Oxaliplatrin: Platin analogon, alkylating agent

Irinotecan: Camptothecin, topoisomerase I inhibitor

Dose: Day1: 400mg/m^2 bolus i.V. followed by 1200 mg/m^2/day as continuous infusion over 46 hours, every 14 days

1.6.2 Individual oncological treatment

In order to identify the most suitable oncological treatment for a patient, the performance status (ECOG) as well as bilirubin levels must be considered.

ECOG performance status:(92)

0 : fully active, able to carry on all pre-disease performance without restriction
1. : restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2. :Ambulatory and capable of all self-care but unable to carry out any work activities; able to move about freely for more than 50% of waking hours
3. Capable of only limited self-care; confined to bed or chair for more than 50% of waking hours
4. Completely disabled, cannot carry out any self-care; totally confined to bed or chair
5. Dead

In order to achieve the best results, combined chemotherapies should be used. If the ECOG performance status or biliary function of a patient do not allow a combination of chemotherapeutics, monotherapies containing only one agent may be used alternatively. Unfortunately, response rates for single agent therapies appear to be less than 10 percent, which means a median survival duration of only 6 to 7 months.(93–95)

1.6.2.1 First line Treatment

The best results concerning overall survival could be reached with the following three **first line therapy** options:(89)

- For patients with an **ECOG performance status of 3 or higher** the only reasonable option seems to be best supportive care.
- For patients with an **ECOG performance status of 2** a combined chemotherapy with gemcitabine and nab-paclitaxel can be considered. If the patient presents with an ECOG status of 2 but shows elevated bilirubin levels (higher than 1,5ULN) a monotherapy with gemcitabine may be started.
- For patients with an **ECOG performance status of 0 or 1** and a low bilirubin level, either FOLFIRINOX regimen or Gemcitabine combined with nab-paclitaxel may be considered. If the bilirubin level is elevated, a chemotherapy without Irinotecan, that is FOLFOX, is suggested.(88)

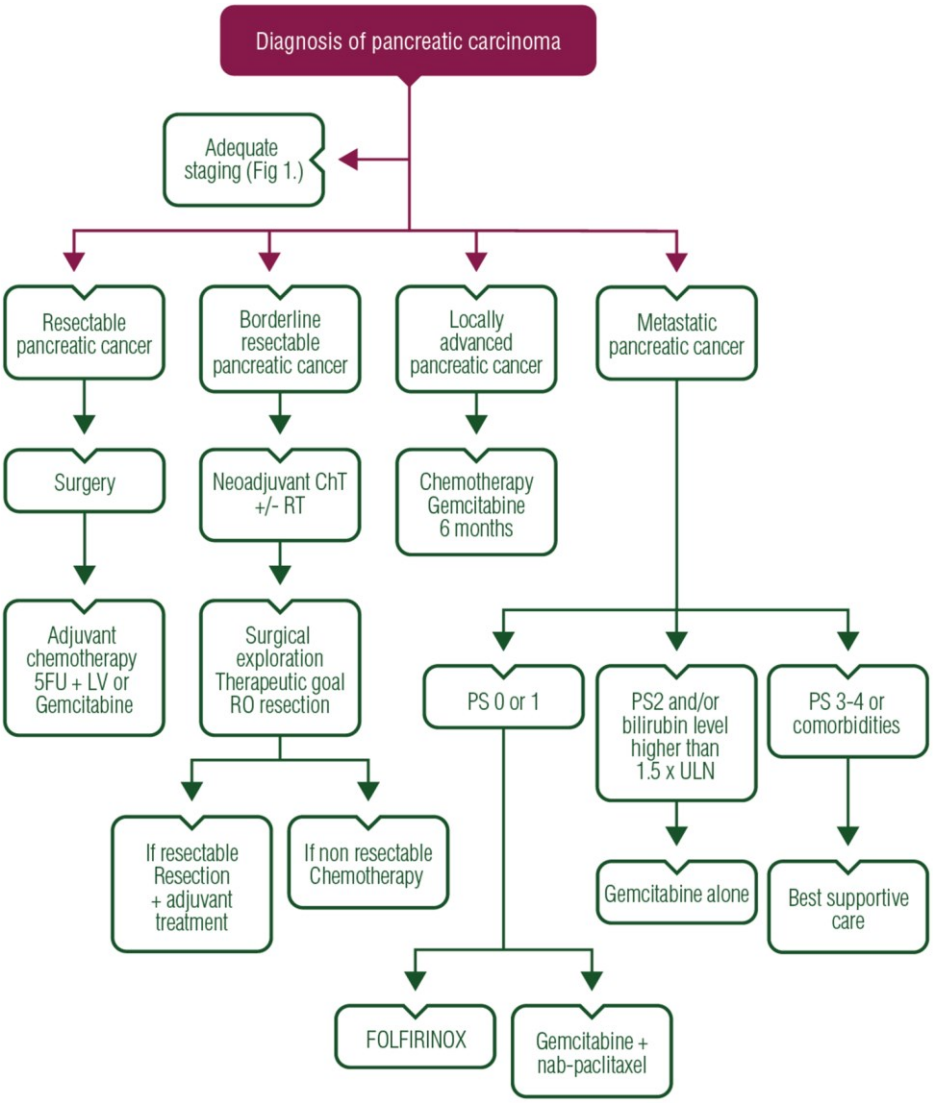

Figure 8: Treatment strategy

ChT, chemotherapy; RT, radiotherapy; 5-fluorouracil; LV, leucovorin; PS, performance status; ULN, upper limit of normal
Adopted by: "Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and folloe-up" by Ducreux M., CuhnaA., et.al., published in 08/2015 in *Annals of Oncology*, volume 26, pages v56-v68

1.6.2.2 Second line treatment

There is no scientifically grounded general guideline for second line therapy of metastatic PC. The individual choice of treatment strongly depends on what the patient received as first line chemo therapy and again on the patient´s ECOG performance status.
According to ASCO (American Society of Clinical Oncology), potential second line therapies could be:(88)

- Patients who received FOLFIRINOX as first line treatment:
  If the ECOG performance status, as well as the bilirubin level, did not elevate under FOLFIRINOX, treatment with Gemcitabine combined with Nab-paclitaxel is

suggested. For patients with increased ECOG performance status number or an abnormal biliary function, Gemcitabine monotherapy, Gemcitabine in combination with Capecitabine or Capecitabine monotherapy may be considered.
Patients with FOLFOX as first line treatment due to elevated bilirubin levels:
The chemotherapy chosen should not contain Irinotecan or Gemcitabine due to their high risk of toxicity.

- Patients who were initially treated with Gemcitabine combined with Nab-paclitaxel or Capecitabine:
  Patients who were initially treated with any Gemcitabine based therapy benefit from a second line therapy with nanoliposomal irinotecan combined with fluorouracil and folinic acid.(96)
  For patients with an ECOG performance status of 0 or1, FOLFOX or FOLFIRI as second line treatment may be appropriate.
  For patients with an ECOG performance status higher than 1, monotherapy with Flourouracil plus Leucovorin or Capecitabine is suggested.(88)

Apart from that, for patients whose tumor presents high microsatellite instability a therapy with Pembrolizumab may be considered.

2 Material and Methods

This retrospective analysis included data from 351 patients with metastatic PC (stage IV), who were treated at the Division of Clinical Oncology, Medical University of Graz, from January 2004 to October 2015. All patients presented with histologically confirmed ductal adenocarcinoma of the pancreas and available clinical records, including the plasma levels of amylase and lipase. These pancreatic enzymes were determined routinely in our pre-diagnostic workflow. Patients with other histological subtypes of PC (e.g. azinar, neuroendocrine or mucinous-cystic neoplasms) were excluded from the study, due to their varying clinical outcome.(97) For deceased patients, dates of death were obtained from the central registry of the Austrian Bureau of Statistics, clinical records or phone calls. Patients' records and information were anonymized and de-identified prior to analysis. All clinico-pathological data was retrieved from medical records at the Division of Clinical Oncology, as well as from pathology records from the Institute of Pathology at the same institution. Since the TNM classification system for PC changed during the study period, tumor stages were uniformly adjusted according to the 7th edition of this system. Other documented clinico-pathological parameters included administration of chemotherapy with gender and age. The levels of pancreatic enzymes were determined in heparin plasma within 1 to 3 days prior to the histologically proven diagnosis (by needle biopsy) and performed as a part of routine clinical practice. Lipase and pancreatic amylase were measured enzymatically (Roche Diagnostics, Mannheim, Germany) on a cobas analyser from Roche.

Treatment decisions were made according to the current ESMO guidelines at the time of treatment. The study was approved by the local ethical committee of the Medical University of Graz (26-196 ex 13/14).(1)

3 Statistical analysis

Cancer-specific survival (CSS) was defined as the timespan from the date of histologically proven diagnosis to cancer-related death (measured in months). The optimal cut-off values for amylase and lipase were determined by applying receiver operating curve (ROC) analysis to differentiate between survival and death (using the MedCalc statistical software version 13.3) as previously described.(98) The association between the pancreatic enzymes and clinico-pathological parameters was evaluated through parametric or non-parametric tests (student T Test, Mann-Whitney U and chi square test). Patients' clinical endpoint was calculated using the Kaplan-Meier method and tested for significance using the log rank test. Univariate and multivariate Cox proportion analyses were performed to determine the influence of different clinico-pathological parameters on CSS. Hazard ratios (HRs) estimated from the Cox analyses were recorded as relative risks with corresponding 95% confidence intervals. All statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). A two-sided $p<0.05$ was considered statistically significant.(1)

4 Results

Within the study cohort, patients had a median age at diagnosis of 66.0 years (interquartile range 57–72 years, minimum 35 years, maximum 85 years) Median levels of CA19-9 were 1191.7 U/ml (interquartile range 177–10,390 U/l; normal range in healthy individuals: 0–37 U/l). Median survival time was 6 months (range, 0–44 months) and 338 (96.3%) of the patients had died by the date of the last follow-up. The mean value determined for amylase was 35 U/l (range 1–561 U/L, normal range in healthy individuals: 15–53 U/l) and 95 U/l for lipase (range 5–1597 U/l; normal range in healthy individuals: 13–60 U/l). An overview of all clinicopathological parameters in this study cohort is shown in *Table 2*.

Table 2: Frequency of clinico-pathological parameters in patients with stage IV pancreatic cancer in this cohort.

Parameter	Number (%)
Gender	
Female	152 (43,3%)
Male	199 (56,7%)
Age	
<65	147 (41.9%)
≥65	204 (58.1%)
Karnofsky Index	
<90	221 (63%)
90-100	130 (37%)
Lipase	
<36 U/L	167 (47,6%)
≥36 U/L	184 (52,4%)
Amylase	
<20 U/L	165 (48,2%)
≥20 U/L	177 (51,8%)

In the study cohort a significant and strong correlation (Spearman correlation) between lipase and amylase levels ($R = 0.821$, $p < 0.001$) was found as well as between amylase and

creatinine (R = 0.132, $p = 0.015$) but no significant correlation between amylase and CA19-9 (R = −0.04, $p = 0.474$) or age (R = −0.046, $p = 0.398$). Regarding the lipase levels, no correlation could be found between lipase and CA19-9 (R = −0.09 $p = 0.108$), age (R = −0.33, $p = 0.543$) or creatinine (R = 0.105, $p = 0.050$). There was also no correlation between amylase and the inflammatory marker Neutrophil-to-Lymphocyte-Ratio (NLR; R = 0.040, $p = 0.587$), lymphocyte count (R = 0.018, $p = 0.768$) or C-reactive protein (R = 0.039, $p = 0.536$) and no correlation between lipase and the inflammatory marker NLR (R = 0.072, $p = 0.324$), lymphocyte count (R = 0.048, $p = 0.438$) or the C-reactive protein (R = 0.039, $p = 0.530$). Furthermore, no association was found between pancreatic enzymes and gender, tumor grade, administration of gemcitabine-based chemotherapy or Karnofsky performance status *(Tables3 and4)*.

Table 3: The relation between clinico-pathological parameters and the pretreatment plasma amylase of patients with stage IV pancreatic adenocarcinoma (n=371).

Characteristics	**Amylase <20 U/L**	**Amylase ≥20 U/L**	***p*-value**
Age at diagnosis (yrs) <65 ≥65	 64 101	 76 101	0.435
Gender Female Male	 70 95	 80 97	0.663
Karnofsky <90 ≥90	 31 39	 98 174	0.204
CA-19-9, U/mL <Median >Median	 62 85	 71 97	1.000
Chemotherapy No Yes	 44 121	 57 119	0.286

Table 4: The relation between clinico-pathological parameters and the pretreatment plasma lipase of patients with stage IV pancreatic adenocarcinoma (n=371).

Characteristics	Lipase <36 U/L	Lipase ≥36 U/L	*p*-value
Age at diagnosis (yrs)			
<65	103	118	0.634
≥65	64	66	
Gender			
Female	77	75	0.33
Male	90	109	
Karnofsky			
<90	64	66	0.634
≥90	103	118	
CA-19-9, U/ml			
<Median	62	73	0.734
>Median	90	96	
Chemotherapy			
No	46	58	0.414
Yes	121	125	

Applying receiver operating curve (ROC) analysis an optimal cut-off level was calculated for amylase at 20 U/l and for lipase at 36 U/l for further prognostic exploration. *Fig.8* shows the Kaplan-Meier curves for CSS and reveals that elevated levels of amylase are a consistent factor for poor prognosis in PC patients ($p = 0.0288$, log-rank test). Similarly, high levels of plasma lipase represent a poor prognostic factor ($p = 0.0287$, log-rank test, *Fig.9*).

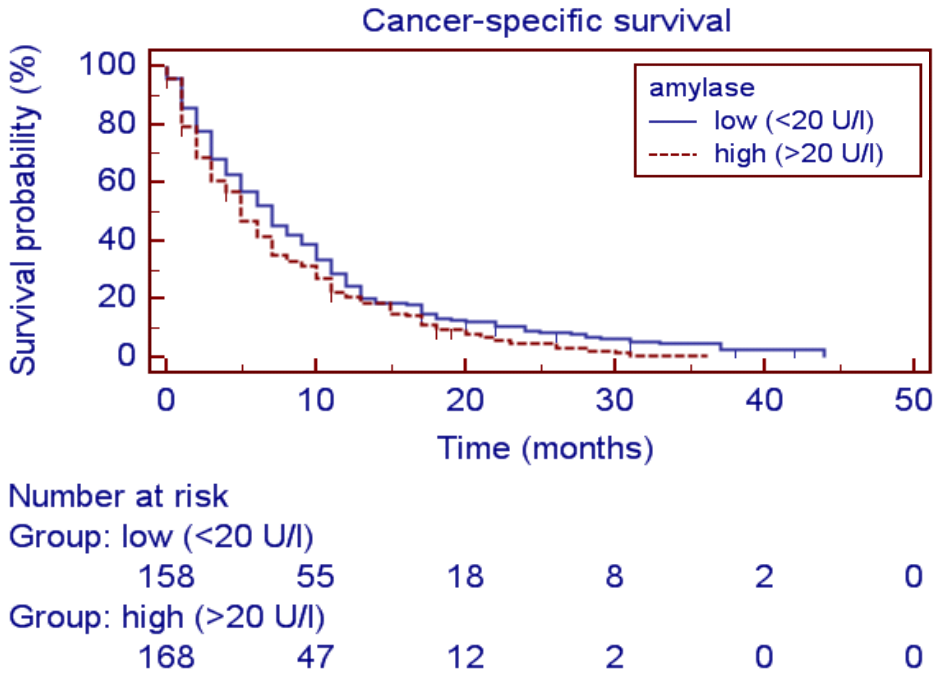

Figure 9: Cancer specific survival: Amylase

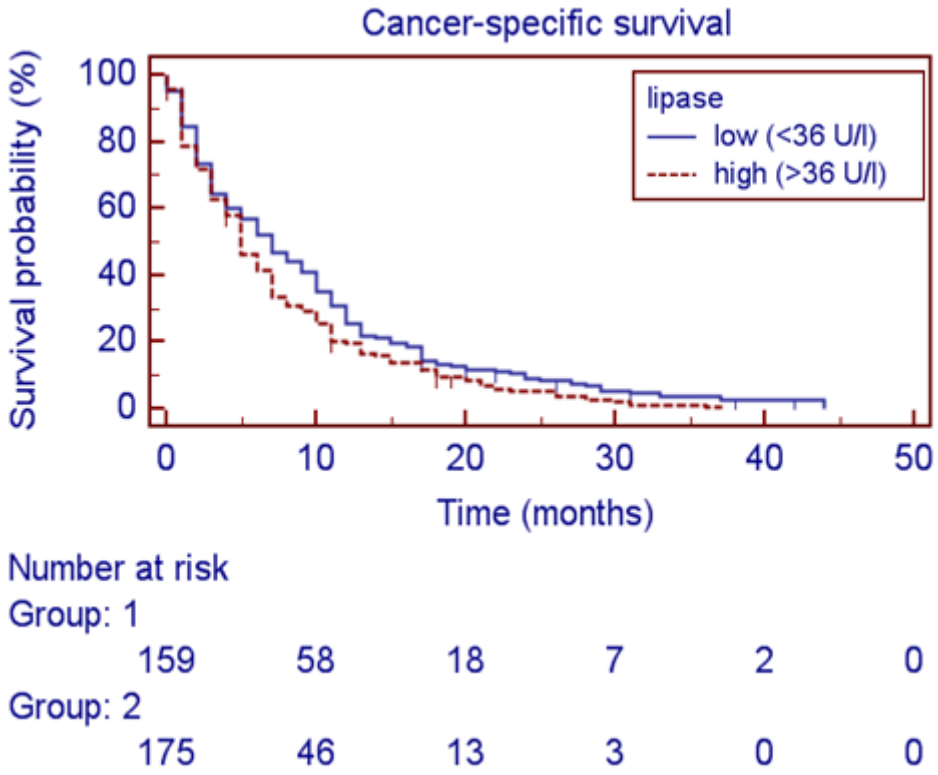

Figure 10: Cancer specific survival: Lipase

To investigate whether the pancreatic enzymes and other clinicopathological factors are associated with the clinical outcome of PC patients, univariate and multivariate Cox proportional models for CSS were calculated. Univariate Cox proportional analysis further identified administration of chemotherapy (no treatment versus chemotherapy, $p < 0.001$), an elevated CA19-9 level ($p = 0.001$), amylase (>20 U/l versus ≤20 U/l, $p = 0.047$), lipase (>36 U/l versus ≤36 U/l, $p = 0.039$) and Karnofsky performance status (<80 versus 90–100, $p = 0.001$) as prognostic factors for poor CSS in this study cohort; however, age (>65 years versus ≤65 years, $p = 0.412$) was not significantly associated with clinical outcome *(Table 5)*. To determine the independent prognostic value of amylase and lipase for CSS, a multivariate analysis using a Cox proportional hazard model was performed. In the multivariate analysis that included age, gender, the Karnofsky performance status and all factors significantly associated with survival in univariate analysis, high levels of CA19-9, administration of chemotherapy and high levels of amylase were identified as independent prognostic factors for CSS (HR = 1.373; 95% CI = 1.004–1.878; $p = 0.047$; *Table 5*).(1)

Table 5: Univariate and multivariate Cox proportional analysis regarding cancer-specific survival

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**
Age at diagnosis(yrs.) Continuous/per year	0.98 (0.99-1.02)	0.412	0.93 (0.73 - 1.19)	0.574
Gender Female Male	1 (reference) 1.07 (0.84-1.34)	0.488	1 (reference) 1.15 (0.90 – 1.45)	0.248
Chemotherapy No Yes	1 (reference) 0.32 (0.242-0.398)	<0.001	1 (reference) 0.32 (0.24-0.43)	<0.001
CA19-9 elevated No Yes	1 (reference) 1.46 (1,16-1.83)	0.001	1 (reference) 1.29 (1.02 – 1.64)	0.035
Amylase <20 U/L ≥20 U/L	1 (reference) 1.29 (1.01 – 1.57)	0.039	1 (reference) 1.40 (1.03 – 1.92)	0.034
Lipase <36 U/L ≥36 U/L	1 (reference) 1.38 (1.01 – 1.56)	0.039	1 (reference) 0.94 (0.69 – 1.23)	0.689
Karnofsky Index <80 90-100	1 (reference) 0.67 (0.54-0.84)	<0.001	1 (reference) 0.88 (0.69 – 1.13)	0.314
Creatinine (Continuous)	1 (reference) 1.00 (0.99 – 1.02)	0.483	1 (reference) 1.01 (0.97 – 1.02)	0.176

5 Discussion

Even considering all of the above, numerous questions about PC still remain unanswered. As for example the reason for the fact, that men have a higher risk for the development of PC. One possible explanation for this phenomenon could be the still higher likeliness of alcohol abuse or smoking among men than among women which could explain it not entirely but could provide a hint.

Regarding the management of metastatic PC, there are only few measures with meaningful impact on the prediction of the survival time of patients. To great extent, an individual patient's prognosis depends not only on the histology of the tumor, but rather, on the extent of its spread (TNM stage). The ductal adenocarcinoma is the most common histological subtype and the prognosis is very poor (99). To keep invasive methods for determination of molecular and genetic prognostic factors to a minimum, circulating biomarkers may offer a promising area for further investigation (100,101). In the present study we were able to verify that there is an association between elevated amylase at the time of diagnosis and poor clinical outcome for patients with metastatic PC.

Amylase has several isoforms, which can be secreted by the pancreas (P form) and the salivary glands (S form) but can also be found in smaller quantities in some other tissue types (102). Amylase is a relatively small molecule (50.000 Daltons) with the main function of breaking down starch into smaller polysaccharides at the internal 1 to 4 alpha linkage during the digestive process (103). The measurement of amylase in plasma is a commonly used routine test, obtained as a biomedical marker for acute pancreatitis (104). There are several other reasons for elevated plasma amylase besides acute pancreatitis. For example, renal failure or more precisely impaired renal clearance may cause an increase of amylase in the blood stream as soon as the creatinine clearance drops below 50ml/min (105). Therefore, we performed a correlation analysis of amylase/lipase and markers of the systemic inflammatory response (CRP, NLR and lymphocyte count) as well as creatinine, but could not find any significant correlation between the inflammatory response markers. However, we found a correlation between the creatinine level and the amylase level. PC Patients with high creatinine levels might not have been the fittest which might had an impact on the survival rate as well.

Moreover, most commonly used amylase assays cannot separate between pancreatic and salivary amylase. Alcoholics for instance may have increased amylase levels of salivary origin, which could create severe difficulties in interpretation when alcoholic abuse is not

yet recorded in the patient´s anamnesis (106). Plasma amylase may also be elevated when amylase is bound to immunoglobulin or polysaccharides building the so called macroamylase which due to its larger size can no longer be filtered by the kidney anymore (107). Under all such circumstances patients typically present with a chronic elevation of amylase. To sum up, the predictive value of plasma amylase is crucially influenced by the clinical context, however, acute pancreatitis is not the only cause.

To return to amylase in association with cancer the question arises why patients with metastatic PC and elevated amylase levels have a poor prognosis. As PC, especially when located in corpus or cauda of the pancreas, remains symptom-free for a considerable period of time, in most cases it has already formed distant metastases at time of diagnosis. It poses a big challenge to determine which of the affected organs is responsible for the elevation of amylase. Whether elevated levels of this biomarker are caused by liver failure due to massive liver metastases, by renal failure due to drugs or prior infections that damage the kidney or by the pancreas itself due to co-infection is unclear in the majority of cases. Therefore we can only speculate about the pathophysiological background underlying these prognostic findings of amylase at the time of diagnosis, as data, regarding any co-morbidity that could have additionally affected the plasma amylase, were limited. Nonetheless we were able to demonstrate that an elevated amylase is associated with a significantly shorter cancer survival time in multivariate analysis.

Based on our findings, the non-invasive measurement of amylase levels at time of PC diagnosis seems to be a new marker for individual patient´s risk assessment and can be regarded as a promising independent prognostic parameter in PC patients.

The strength of this study is the large sample size and the long follow–up period. However, due to the retrospective design of the study, we cannot fully exclude a selection bias in our study cohort. Even though, the prognostic value of amylase determination in daily practice is perhaps limited as we only explored these significant results in advanced tumor stages and because the prognosis of PC patient´s survival duration is poor in general.

In conclusion, our study is the first to indicate that increased amylase levels are a negative prognostic marker in PC patients.(1)

6 Conclusion

There are only few measures with meaningful impact on the prediction of the survival time of PC patients; however, circulating biomarkers tend to represent a promising area for investigation. In the present study we were able to verify that there is an association between elevated amylase at the time of diagnosis and poor clinical outcome for patients with metastatic PC. The measurement of amylase in plasma is a commonly used routine test, which means it is a low cost, easy and non-invasive option to stratify patients into different risk groups in clinical trials and clinical decision-making.

7 Acknowledgement

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