Diplomarbeit

Liver enzymes and synthesis parameters as prognostic factors in pancreatic cancer.

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Graz, am 11.06.2019 *Eva Valentina Klocker eh*

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Zusammenfassung

Hintergrund

Prognosemarker spielen im Rahmen von Therapieentscheidungen eine wichtige Rolle. Das Pankreaskarzinom stellt mit einer 5 Jahresüberlebensrate von 9-10% einen hochmalignen Tumor dar, zumal es aufgrund unspezifischer Symptome häufig erst zu einer Diagnosestellung in ausgedehnten Stadien kommt. Wir untersuchten Leberenzyme wie Aspartat-Aminotransferase (AST), Alanin-Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT) und Alkalische Phosphatase (AP) sowie Bilirubin und Lebersyntheseparameter wie Cholinesterase (CHE) und Thromboplastinzeit.

Methoden

In dieser retrospektiven Datenanalyse wurden prädiagnostische Laborparameter von 547 Patientinnen und Patienten mit histologisch bestätigtem Pankreaskarzinom untersucht. Der primäre Endpunkt war das tumorfreie Überleben.

Ergebnisse

In der multivariaten Analyse zeigten sich eine Cholinesterase <7272U/L (p-value = 0.006) sowie ein Bilrubin-Spiegel ≤1.2mg/dL (p-value = 0.037) und ≤1.9mg/dL (p-value = 0.037) als unabhängige Prognosemarker für ein schlechteres tumorfreies Überleben.

In der Kaplan-Meier Analyse zeigte sich ein signifikant verlängertes tumorfreies Überleben bei Bilirubin-Spiegeln von ≤1.2mg/dL und ≤1.9mg/dL, bei einer Gamma-Glutamyltransferase ≤25U/L, einer Alanin-Aminotransferase >64U/L, einer alkalische Phosphatase ≤70U/L sowie einer Cholinesterase-Aktivität >7272U/L.

Conclusio

Diese Studie zeigt die Rolle von Leberenzymen und Lebersyntheseparametern als mögliche Prognosemarker im Pankreaskarzinom. Aufgrund der großen Variabilität die geeigneten Grenzwerte im Vergleich mit anderen Studien betreffend, erscheinen weitere größere Studien erforderlich.

Abstract

Background

Prognostic markers play in important role in decision making for tumour therapy. We evaluated liver enzymes such as aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) and bilirubin and liver synthesis parameters such as cholinesterase (CHE) and prothrombin time (PT) in patients with pancreatic cancer.

Methods

Our retrospective data analysis included data from 574 patients with histologically confirmed pancreatic cancer. The primary endpoint was cancer-specific survival analyzed by Kaplan-Meier method. Additionally, univariate and multivariate Cox analysis was performed.

Results

Multivariate analysis revealed CHE <7272 U/L (p-value = 0.006) and bilirubin values ≤1.2mg/dL (p-value = 0.037) and ≤1.9mg/dL (p-value = 0.037) as independent markers of poor prognosis. Kaplan-Meier analysis showed a significantly longer cancer-specific survival in association with bilirubin values of ≤1.2mg/dL and ≤1.9mg/dL, a GGT ≤25U/L, an AST of >64U/L, an ALP of ≤70U/L and a CHE of >7272U/L.

Conclusio

Our study showed liver enzymes and liver synthesis parameter as possible prognostic markers. However, more studies seem necessary, particularly in order to establish useful cut off values for the investigated parameters.

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Abbreviations

AJCC……American Joint Committee on Cancer
ALP……alkaline phosphatase
ALT……..alanine aminotransferase
APTT……activated partial thromboplastin time
AST……aspartate aminotransferase
ASCO……....American Society of Clinical Oncology
AT-III……antithrombin-III
BUN……..blood urea nitrogen
Ca 19-9……...carbohydrate antigen 19-9
CI……...confidence interval
CHE……...cholinesterase
CT……computed tomography
CSS……cancer specific survival
DD……...D-dimer
dMMR……deficient mismatch repair
ECOG PS……Eastern Cooperative Oncology Group Performance Status
ERCP……..endoscopic retrograde cholangiopancreaticography
EUS-FNA ……endoscopic ultrasound guided fine-needle aspiration cytology
F……...fibrinogen
FAMMM……..familial atypical multiple mole melanoma syndrome
FAP……familial adenomatous polyposis
FNA……fine needle biopsy
FPC……..familial pancreatic cancer
F-VIII…….....factor VIII
GGT……gamma-glutamyl transferase
HNPCC……..hereditary nonpolyposis colorectal cancer
HR…….hazard ratio
INR……....international normalized ratio
IPMN……...intraductal papillary mucinous neoplasm
ITPN……..intraductal tubulopapillary neoplasm
LAPC……locally advanced pancreatic cancer
MCN……...mucinous cystic neoplasm
MRCP……magnetic retrograde cholangiopancreaticography
MRI…….magnetic resonance imaging
MSI-H……...microsatellite instability-high
PanIN……pancreatic intraepithelial neoplasia
PC……..protein C
PT…….prothrombin time
PNET……...primitive neuroectodermal tumor
SPEN…….solid pseudopapillary mucinous neoplasm

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1 Introduction

1.1 Definition

Pancreatic cancer is one of the most aggressive forms of cancer. Accordingly, an effective screening method would seem highly desirable, but is currently not yet available. In general, the developing pancreas carcinoma is not associated with the expression of early symptoms, an exception being cases where the tumors are located in the area of the papilla duodeni vateri. As a result, pancreatic cancers are often detected in locally advanced or metastasized stages at the time of diagnosis. Thus, although, numerous new antitumor medications with various mechanisms of action have been well established over the last years, treatment of pancreatic cancer still follows rather simple and standardized protocols when compared to other cancer entities and the prognosis is in general still very poor.

1.2 Pathogenesis

Both anatomically and physiologically the pancreas consists of an exocrine and an endocrine part and pancreatic cancer may occur within either of both parenchyma. Nonetheless, 95 percent of all pancreatic carcinomas arise from the exocrine tissue, particularly from acinar cells in the ductal epithelium or connective tissue. (1) In more than 90% of pancreatic cancers a mutation of KRAS is found. (2) However, lots of other mutations seem to be important for the development of cancer. Considering that precursor lesions of pancreatic cancer could be found in normal pancreatic tissue, it is obvious to assume that tumorigenesis is a sequence of mutations leading to the development of pancreatic cancer. Mutations of K-RAS can be already been found in PanIN-1- lesions and the incidence of this mutations correlates with the grade of dysplasia. (3) These pancreatic intraepithelial neoplasia are microscopic lesions with a diameter of less than 5mm and can be divided in three grades depending on their grade of dysplasia. In combination with other effector protein signaling pathways such as the RAF-MEK-ERK/MAPK-signal cascade, K-ras activates cyclin D. (4) Cyclin D, in turn, acts on RB, a major effector protein of the cell cycle. (4) PanIN-2- lesions may show an inactivation of CDKN2 and PanIN-3-lesions present mutations in the tumor-suppressor genes TP53 and DPC4/SMAD4. (4)(5) Deletion

of DPC4 occurs late in tumorigenesis and leads to infiltrative growth. (6) Mutations in GNAS1 and KRAS seem to play a role in the early development of IPMN whereas TP53 mutations occur in the advanced stages. In summary, it has been hypothesized that normal duct becomes PanIN-1A (flat duct lesion) and PanIN-IB (papillary duct lesion) by mutations in Her-2/neu or K-ras. (6) Mutations in p16 may lead to PanIN-2 (atypical duct lesion) and mutations in p53, DPC4, BRCA2 to PanIN-3 (carcinoma in situ). (6)

It has been hypothesized that because of the fact that pancreatic carcinomas build a prominent stroma around themselves their response to chemotherapy is rather poor. (7) However, the exact mechanism underlying this phenomenon has not yet been fully understood. (7)

1.3 Histology and precancerous lesions

85 percent of pancreas carcinomas are pancreatic ductal adenomcarcinomas. (8) The main precurser lesion typically observed is the pancreatic intraepithelial neoplasia (PanIN). (9) However, there are also other precursor lesions referred to as intraductal papillary mucinous neoplasm (IPMN) and the mucinous cystic neoplasm (MCN) which appear as macroscopic cysts. (9)

IPMN show an intraductal growth in mucin-producing main pancreatic duct or of one its branches and produce mucin. (10) IPMN located in the branches tend to appear mulitlocated in the processus uncinatus and are associated with familial pancreatic cancer. (11) These lesions are similar to the foveolar epithelium of the gaster- so they are of the gastric type- and have low malignant potential. IPMN from the main pancreatic ducts prodominate in the pancreatic head and often they are assocaited with an invasive carcinoma at time of diagnosis. (11) These IPMN from the main pancreatic ducts can be categorized into intestianal, pancretobilary and oncocytic types. In comparison, MCN grow intraparenchyma and can be described histologically as a mucinous epithelial lining. (10)

Table 1. Histological types in pancreatic cancer. Modified after Steinman et al. (8)

Histological type	Incidence	Median age (years)	male:female ratio
Ductal adenocarcinoma	85%	68-72	1:3

Serous cystadenoma	1-2%	58	1:3
Mucinous cystic neoplasm (MCN)	1-2%	45	1:20
Intrapapillary mucinous neoplasm (IPMN)	3-5%	67	1:1
Neuroendocrine tumor (PNET)	3-4%	59	1:1
Metastases	2-5%	59	2:1
Solid pseudopapillary mucinous neoplasm (SPEN)	1-2%	22	1:10
Acinar cell carcinoma	1%	61	4:1
Pancreatoblastoma	<1%	5	1:1
Lymphoma	<0.5%	63	7:1
Lymphoepithelial cyst	<1%	56	4:1
Lipoma	<1%	66	1:1
Lymphangioma	<1%	40	1:2
Sarcoma	0.1%	57	1:1
Neurogenic tumor	<0.1%	Variable	1:1
Intraductal tubulopapillary neoplasm (ITPN)	<0.1%	61	1:1
Teratoma	<0.1%	40	1:1

Alternatively, it is possible to categorizes malign pancreatic lesions by their appearence in the CT in hypoenhancing, hypenhancing, and cystic neoplasm, though. (8)

1.3.1 Metastasis

Metastasis in the pancreas are not very rare. However the most common cancers to metastasize to the pancreas are malignancies of the lung, the small cell broncus carcinoma, the gastrointestinal tract, the kidney and the breast. (12) Melanomas and hematopoietic tumors metastasize to the pancreas as well. (12) Their enhancement depends on their site of origin. (8)

1.4 Classification

1.4.1 TNM Staging as AJCC Cancer Staging Manual 8th edition (13):

1.4.1.1 T Category:

TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
Tis Carcinoma in situ. This includes high-grade pancreatic intraepithelial neoplasia (PanIn-3), intraductal papillary mucinous neoplasm with high-grade dysplasia, intraductal tubulopapillary neoplasm with high-grade dysplasia, and mucinous cystic neoplasm with high-grade dysplasia.
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 1–2 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

T (m) Selected if synchronous primary tumors are found in a single organ

1.4.1.2 N Category:

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

N (sn) Selected if regional lymph node metastasis identified by SLN biopsy only.

(f) Selected if regional lymph node metastasis identified by FNA or core needle biopsy only

1.4.1.3 Category M:

M0 No distant metastasis

cM1 Distant metastasis

pM1 Distant metastasis, microscopically confirmed

1.4.2 Staging:

Table 2. TNM Staging. Modified after Amin et al.(13)

T	N	M	Stage
Tis	N0	M0	0
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

Cancers of the pancreatic head are located on the right side of the vena mesenterica superior. The processus uncinatus is considered to be part of the pancreatic head as well. Pancreatic carcinomas tend to metastasize to lymph nodes located near the ductus choledochus, arteria hepatica communis, vena porta and near pyloric, infrapyloric and subpyloric lymph nodes. Furthermore, they may metastasize to mesenterial, coeliacal, anterior and posterior pancreaticoduondenal lymph nodes and along the arteria mesenterica superior. (14)

In general, carcinomas are referred to as cancers of the pancreatic corpus when they grow between left to vena mesenterica superior and the left to the aorta. (14) Cancers of the pancreatic tail approach between the left side of the aorta and the hilus of the spleen. (14) Both tend to metastasize to the lymph nodes along the arteria hepatica communis, coeliacal lymph nodes and along the spleen artery, the hilus of the spleen, retroperitonal, lateral aortal. (14)

1.5 Epidemiology

The pancreas carcinoma is known as one of the most aggressive tumors. In Austria in 2016 1799 people were diagnosed with pancreatic cancer. (15) With age standardization the incidence was 21 per 100000 with a death rate of 20 per 100000. (15)

The 1799 people with pancreatic cancer in Austria in 2016 corresponded to 4 percent of all malign tumor diseases observed. Due to its lethality, it was the third cause of death from cancer in Austria. (15) From 2014 to 2016 one third of pancreatic cancers were in an advanced stage at time of diagnosis and only 22 percent were in a local stadium. (15) However, in about 40 percent of all cases there was not any clear diagnosed stadium documented, including DCO cases. (15) From 2014 to 2016 the relative survival rate for one year was 38 percent and the relative survival rate for five years (2009-2013) amounted

to 10 percent. (15) In women the relative 5 year survival rate was slightly higher (9.6 percent to 10.1 percent). (15)

In the United States, the 5 year-survival- rate between 2008 and 2014 was 9 percent in pancreatic cancer patients. In local stages the 5-year-survival rate was reported to be 34 percent, in regional advanced stages it was 12 percent and in patients with distant metastases it was only 3 percent. (16)

1.6 Risk factors

As mentioned before, higher age and male gender seem to be risk factors for developing pancreatic cancer. The risk is aggravated in smokers and persons with a positive family history. (17) (18) (19) Furthermore, heavy alcohol consumption, particularly consumption of beer, seems to have an impact of pancreatic cancer risk. (18) The risk of pancreatic cancer is increased in current smokers as well as in former smokers with an odds ratio (OR) of 1.74 (95% CI 1.61–1.87) and 1.2 (95%CI 1.11–1.29) as reported in their meta-analysis by Iodice et al. (19) Lynch et al. showed that pancreatic cancer risk increases with duration of smoking (> 50years: OR 2.13, 95%CI 1.25 to 3.62, p-value: <0.001), smoking intensity (≥ 30cigarettes per day: OR 1.75, 95%CI 1.27-2.42,p-value=0.03) and cumulative dose defined as pack years (>40py: OR 1.78, 95%CI:1.35-2.34, p-value <0.001) compared to never smokers. (20)

Although diabetes mellitus is reported as risk factor for pancreatic cancer, it also seems to be an early sign for this malignancy. Furthermore, diabetes mellitus can be a result of pancreatic insufficiency experienced as a consequence of pancreatic cancer. Therefore, a bidirectional relation can be assumed. However, the mechanism remains still unclear. A meta-analysis of 36 studies published in 2005 reported a summary OR of 1.82 (95%CI 1.66-1.89) in patients with diabetes mellitus type II. (21) In a case control study in Italy the risk of pancreatic cancer was increased 2.89-fold (95%CI 1.71 to 4.86) in patients with diabetes. (22) In this study a difference in risk depending on the used antidiabetic treatment was reported. (22) Compared to participants without diabetes the OR was 1.5 (95%CI 1.1-

2.1) as reported in a population-based-case-control study by Wang et al. (23) Furthermore, a decrease in risk in association to duration of diabetes was documented. (23)

The role of other life style factors such as obesity, diet and physical activity as risk factors is not yet clearly established. (24) (25) (26) (27) There is evidence that some agents may increase the risk of pancreatic cancer in workers such as chlorinated hydrocarbon compounds and insecticides. (28) It seems that the highest risk is observed in laundry and dry cleaning workers and metal-plating workers. (28)

1.6.1 Hereditary factors

Approximately 10 percent of pancreatic cancers appear due to genetic predisposition (29) (30), which is reflected in the fact that between 5 to 10 percent of patients have primary relatives with cancer of the pancreas.

For the diagnosis of familial pancreatic cancer (FPC) it is required that there are two or more primary relatives with pancreatic cancer and that there is no evidence for a familiar pancreatic cancer syndrome. The median age at diagnosis is about 65 years. (31)

Cancer family syndromes include FAMMM syndrome, HNPCC syndrome, hereditary breast and ovarian cancers, Peutz-Jeghers syndrome, the familial adenomatous polyposis, Li-Fraumeni syndrome and ataxia telangiectasia.

1.6.1.1 Hereditary pancreatitis

Patients with hereditary pancreatitis present with chronic inflammation of the pancreas including symptoms such as abdominal pain usually located in the epigastric region with radiation to the back, vomiting and nausea. Due to chronic inflammation complications like fibrosis, calcifications and obstruction of the biliary duct are not infrequent and may lead to pancreatic insufficiency. Mutations in the PRSS1, CTRC, SPINK1 and CFTR gene

have been reported. (32) However, there may be many more not yet detected mutations responsible for hereditary pancreatitis.

In patients with hereditary pancreatitis the cumulative risk to develop pancreatic cancer at the age of 70 years is 40 percent, although this is associated with a rather broad confidence interval (95% CI 9-71%). (33) A better predictive factor seems to be a paternal inheritance pattern. In these parents the cumulative risk was shown to be elevated by up to 75 percent (95% CI 32-100%) at the age of 70 years. (33) Considering the large confidence interval in association with age it appears likely that there may be other essential factors that influence the risk of pancreatic cancer development.

1.6.1.2 FAMMM syndrome

The Familiary Atypical Multiple Mole Melanoma syndrome is transmitted in an autosomal dominant fashion and is associated with mutations in the CDKN2A and CDK4 genes which lead to atypical moles, increased occurrence of melanomas and pancreatic cancers. The cumulative risk in patients with a 19 bp deletion in exon 2 of p16 gene until the age of 75 years adds up to 17 percent. (34)

1.6.1.3 HNPCC syndrome

The Hereditary non-polyposis colorectal cancer syndrome, also referred to as Lynch syndrome, presents mutations in genes that are important for DNA repair, such as MSH1, MLH1, MSH6, PMS1 or PMS2. The relative risk to develop pancreatic carcinoma is elevated 9 to 11 fold compared to the general population. (35)

1.6.1.4 Hereditary breast and ovarian cancer

These syndromes present mutations in the tumor suppressor genes BRCA 1 and 2. The risk of pancreatic cancer is increased 2.6-fold in BRCA 1 mutations and 2.1-fold in BRCA2

mutations. (35) Although BRCA 2 is an accepted risk factor for developing pancreatic cancer, the role of BRCA 1 in pancreatic cancer remains discussed. (36) Mutations in PALB 2 are associated with an increased risk of pancreatic cancer beside previous mentioned mutations as well. (29)

1.6.1.5 Peutz- Jeghers syndrome

Patients with this autosomal dominant inherited syndrome will present with hamartomas localized in the gastrointestinal tract and pigmentations on the oral mucosa and in the face perioral. (37) (35) In patients with Peutz-Jeghers syndrome mutations in the STK11 (Serin-Threonin-Kinase 11) gene can be found by an increased relative risk of pancreatic cancer up to 132fold. (38) (35)

1.6.1.6 FAP syndrome

The familial adenomatous polyposis is inherited in an autosomal dominant way and shows a defect of the APC gene. Patients with familial adenomatous polyposis present with multiple polyps in the colon which tend to progress to malign lesion over time. Although there is an elevated risk to develop colon cancer the risk for other malignances is higher compared to the general population. The relative risk to develop pancreatic cancer is reported to be increased 4.5-fold. (35)

1.6.1.7 Ataxia teleangiectasia

Ataxia teleangectasia may be associated with the appearance of pancreatic cancer. The life time risk not determined yet. (39)

1.7 Clinical presentation

Patients with pancreatic tumors may present unspecific symptoms. Pancreatic tumors usually remain undetected for quite a long time and provoke clinic symptoms by invading collateral structures, such as for instance vessels, nerves or the biliary duct or by metastasizing to other distant organs. (40) Typical clinic signs comprise abdominal pain ache in the middle of the back, weight loss and obstructive jaundice. (40) Other clues for pancreatic cancer include new-onset diabetes and thrombembolic events. (40)

The most frequent symptoms at presentation with pancreatic cancer were reported in a multi-institutional analysis with asthenia associated with 86 percent, weight loss with 85 percent, anorexia with 83 percent, abdominal pain with 79 percent, epigastric pain with 71 percent, dark urine with 59 percent, jaundice with 56 percent, nausea with 51 percent, back pain with 51 percent, diarrhea with 44 percent, vomiting with 33 percent, steatorrhea with 25 percent and thrombophlebitis with 3 percent. (41)

It has been reported that 60 to 70 percent of pancreatic cancers are located in the pancreatic head, whereas 20 to 25 percent arise in the body and tail and only about 10 to 20 percent grow diffusely in the pancreas. (1) However, the clinic manifestation critically depends on the tumour location. Carcinomas in the pancreatic head are more likely to cause jaundice and steatorrhea than cancers in the tail or body. (41) (1) Courvoisier´s sign is reported as a clinical symptom in 13 percent of pancreatic cancer patients. (41) It describes a palpable but not painful gallbladder. Another clinic manifestation of pancreatic cancer may be acute pancreatitis. (42)

A new-onset diabetes or a new-onset glucose intolerance is a possible early indication for pancreatic cancer. (43) (44) There may be also a temporal association to the diagnosis. Chari et al showed, that in patients over 55 years developed pancreatic cancer is more likely to be detected within 3 three years after diagnosis of new-onset diabetes in comparison to the general population. (44) About 80 percent of patients with pancreatic cancer show impaired glucose tolerance or diabetes. (43) Diabetic symptoms associated with pancreatic cancer tend to improve after tumour resection whereas long-standing diabetes remains unaltered. (45) However, the association seems to be bidirectional and is not fully clarified yet. (23)

1.7.1 Paraneoplastic phenomenon

Pancreatic cancer provokes the expression of different paraneoplastic symptoms such as hypercoagulability, thombolphlebitis migrans, thrombophilia and panniculitits nodularis. (46) (47) (40) The incidence of thromboembolic events including deep vein thrombosis, pulmonary embolism, portal vein thrombosis, disseminated intravascular coagulation and arterial thromboembolism varies between 17 and 57 percent. (48) The mechanism of hypercoagulability in pancreatic cancer is not yet clearly understood, but there is evidence that patients with pancreatic cancer present higher levels of F-VIII, fibrinogen, D-dimer, tissue factor, thrombin-antithrombin III complex and cystein proteinase cancer procoagulant. (46) Additionally, decreased levels of protein C and antithrombin III were detected. (46)
However, panniculitits in combination with polyarthritits is a clinic manifestation of pancreatitis, although there is evidence of occurrence as paraneoplastic symptom in pancreatic cancer. (47)

Considering numerous studies that have shown a bidirectional association between new-onset diabetes or impaired glucose tolerance and pancreatic cancer, diabetes or glucose intolerance could be a paraneoplastic symptom. (46)

Aconthosis nigricans is described as another possible paraneoplastic phenomenon in pancreatic cancer which presents with hyperpigmented thickened skin, particularly in intertriginous locations. (46) (49) Furthermore there are other described cutaneous and melanocytic paraneoplastic phenomena such as sign of Leser-Trelaut, necrolytic migratory erythema, paraneoplastic pemphigus, dermatomyositits, erythema nodosum, palmar fasciitis and bilateral diffuse uveal melanocytic proliferation. (46) Less frequently appearing associated diseases are atypical, rapid onset rheumatoid arthritis, polymyositis, dermatomyositis, gastropareses, opsoclonus and nephritic syndromes. (46)

1.7.2 Metastasis

Pancreatic cancer tends to most frequently metastasize to the liver, followed by the peritoneum. Other locations for metastasis are the lungs, adrenal glands, abdominal lymph

nodes or bones. (50) Depending on the exact site of metastases, additional symptoms are provoked in metastatic pancreatic cancer.

1.8 Diagnosis

Considering the abovementioned unspecific symptoms precise diagnostics appear an essential requirement. It is therefore advised to test in all patients with jaundice, epigastric pain and weight loss laboratory parameters such as aminotransferases, alkaline phosphatase, bilirubin and in acute pancreatitis also lipase.

1.8.1 Laboratory values, Ca 19-9

Ca 19-9 is an established tumor marker for follow-up after the initial diagnosis of potential pancreatic cancer. However, it is not a suitable selective diagnostic marker because it is also expressed in other types of cancer and in benign diseases, specifically with obstructive jaundice. Ca 19-9 is reported with a sensitivity of 70 percent and a specificity of 87 percent, the negative predictive value amounting to 92 percent, the positive predictive value to 59 percent. (51) However, Teng et al. showed that significantly elevated Ca 19-9 levels may indicate the presence of pancreatic cancer in patients with acute pancreatitis. (52)

1.8.2 Sonography

Transabdominal sonography is indicated in jaundice to detect an obstruction of the biliary duct. However, by transabdominal ultrasound only tumors larger than 3cm can be detected. The sensitivity of the method is about 83 to 89 percent for detecting pancreatic cancers with a specificity of about 99 percent. (53) (54)

1.8.2.1 Endoscopic ultrasound

A Cochrane metaanalysis reported a sensitivity of endoscopic ultrasound examination 0.95 (95% CI 0.84 to 0.99) and a specificity of 0.53 (95% CI 0.31 to 0.74) to differentiate malign versus benign pancreatic lesions. (55) The accuracy of the method to distinguish between invasive lesions and dysplasia had a sensitivity of 0.78 (95% CI 0.44 to 0.94) and a specificity of 0.91 (95% CI 0.61 to 0.98). (55)

1.8.3 Computed tomography

In the abovementioned Cochrane analysis the sensitivity and specificity for differentiating invasive cancer from precancerous lesions by CT was reported to be 0.72 (95% CI 0.50 to 0.87) and 0.92 (95% CI 0.81 to 0.97), respectively. (55) Vincent et al consider tri-phasic pancreatic-protocol CT as the best initial diagnostic tool with an accuracy of 80 percent to predict the resectability of the cancerous lesion. (40) However, the accuracy of detecting pancreatic cancer depends on the size of the tumour. In multiphasic helical CT the sensitivity is 72 percent and the specificity 100 percent in tumours smaller than 2 cm in pathological measurements. (56)

1.8.4 Magnetic resonance imaging

Magnetic resonance imaging is usually used in second-line in uncertain cases. However, it was reported to possess a sensitivity of 93.5% (96% sensitivity in CT) and a specificity of 78.9% vs. 86.8% in computed tomography. (57)

1.8.5 Endoscopic ultrasound guided fine-needle aspiration cytology

The sensitivity of endoscopic ultrasound guided fine-needle aspiration cytology for detection of pancreatic cancer is about 80 percent. (58) The specificity of EUS-FNA is reported with 98%. (59) In the Cochrane metaanalysis in 2017 EUS-FNA was recorded

with a sensitivity of 0.79 (CI 0.07 to 1.00) and a specify of 1.00 (CI 0.91 to 1.00) in distinguishing benign and cancerous lesions. (55) The ability to differentiate benign from precancerous and cancerous lesion is given with a sensitivity of 0.73 (CI 0.01 to 1.00) and a specificity of 0.94 (CI 0.15 to 1.00). (55) The sensitivity and specificity for differentiating an invasive cancer versus a precancerous lesion were reported with 0.66 (CI 0.03 to 0.99) and 0.92 (CI 0.73 to 0.98). (55)

1.8.6 Percutaneous biopsy

A biopsy of pancreatic lesions is not recommended. Instead a primary surgery if possible is preferred because it is part of therapy. (40)

1.8.7 Endoscopic retrograde cholangiopancreaticography

Endoscopic retrograde cholangiography should be conducted to exclude choledochlithiasis or for placement of a biliary stent in obstruction of the biliary system. In a systematic review comparing endoscopic retrograde chalangiopancreaticography with brush cytology or forceps biopsy to endoscopic ultrasound guided fine-needle aspiration cytology in diagnosis of malignant biliary structures a sensitivity of 49 percent versus 7 percent and a specificity of 96,33 percent versus 100 percent were reported. (60)

1.8.8 Magnetic resonance cholangiopancreaticography

Magnetic resonance cholangiopracreaticography is reported as diagnostic tool with an accuracy similar to endoscopic retrograde pancreaticography. Furthermore, this method does not require contrast material and is a non invasive method. However, in a prospective study the sensitivity was 84 percent and the specificity was 97 percent versus 70 percent and 94 percent in endoscopic retrograde cholangiopancreatography. (61)

1.9 Screening

At present, there are no established screening tools for pancreatic cancer development. The incidence of pancreatic cancer is very low in the general population with a life time risk of 1.3%. (62) In patients of a specific risk population there are a few studies such as the Cancer of the Pancreas Screening 2 and 3 trials, aiming at developing an appropriate screening by testing EUS, MRI and CT. (63) (62)

1.10 Therapy

By the time of primary diagnostic, the pancreatic cancer is only in 15-20 percent of the patients in a resectable stage. (15)

1.10.1 Therapy modalities

1.10.1.1 5-Fluorouracil

The antimetabolite 5-Fluoruracil which was developed in the 1950s is an analogue of uracil. (64) 5-FU is mostly catabolized in the liver by dihydropyrimidine dehydrogenase (DHD). (65) A genetic deficiency may lead to a fatal toxicity of 5-FU. Considering the variety of genetic mutations not yet discovered, the use of pre-therapeutic screening is still under debate. (65) Fluoruracil is generally administered in combination with leucovorin.
A list of common adverse events associated with 5-Fluorouracil treatment:

- Nausea
- Emesis
- Mucositis
- Diarrhea
- Hand-foot-syndrom
- Dermatitis
- Alopecia
- Myelosuppression

1.10.1.2 Oxaliplatin

Oxaliplatin is as cisplatin platinum compound and inhibits DNA replication as an alkylating agent. Common adverse events caused by this compound are:

- Diarrhea
- Nausea
- Myeolosuppression
- Neuropathy

1.10.1.3 Irinotecan

Irinotecan exerts its effect as an inhibitor of topoisomerase I. It is converted to its active metabolite SN-38. In its nanoliposomal form the conversion to SN-38 proceeds at a slower pace. Due to the risk of cholinergic syndrome irinotecan is given in combination with atropine. Possible adverse symptoms are:

- Cholinergic syndrome
- Myeolosuppression
- Liver toxicity
- Kidney toxicity
- Colitis
- Ileus
- Allergic reaction

1.10.1.4 Gemicitabine

Gemictabine is a nucleosid analog that is phosphorylated in the cell, replaces cytidine as building block for DNA synthesis and thereby inhibits DNA synthesis and induces apoptosis. Possible adverse events are:

- Myelosuppression
- Fatigue
- Nausea

- Emesis
- Elevation of liver specific laboratory values

1.10.1.5 Nab-Paclitaxel

Paclitaxel inhibits cell mitosis as it blocks the degradation of microtubular structures. As nanoparticle albumin bound paclitaxel (nab-Paclitaxel) it leads to a better distribution in body. Common adverse effects after its administration are:

- Myelosuppression
- Fatigue
- Diarrhea
- Neuropathy
- Alopecia

1.10.1.6 Erlotinib

Erlotibinib is an EGFR-tyrosine kinase inhibitor. The most common side effects are:

- Rash
- Diarrhea

1.10.1.7 Surgery

In the light of current clinical data it appears essential for the therapeutic outcome to remove the entire cancerous tissue. (66)

For pancreatic cancer located at the pancreatic head the classic Whipple procedure is advised as well as the pylorus-preserving pancreaticoduodenectomy, the so called pp-Whipple procedure. (67)

The most widely practiced procedure is with an end-to side duct-to-mucosa technique. (68) In this surgical procedure the pancreatic head and sometimes the pancreatic body is removed as well as a part of the small intestine, the bile duct, the gall bladder, regional

lymph nodes and a part of the stomach. By pylorus-preserving pancreaticoduodenectomy the whole stomach remains intraabdominal. The residues of pancreas and bile duct tissue are connected to the small intestine so that the digestive enzymes may be delivered into the small intestine. (66) Usually Whipple surgery is performed through a big abdominal incision. There is also the possibility to do this laparoscopically. Considering the complexity of this procedure the risk to die due to the surgical procedure or as a result of subsequent complications is high. The American Cancer Society reported that 5 to 15 % of patients die depending on the surgeon´s experience. (66)
Possible complications occurring in this context are:

- Infections
- Bleeding
- Leaking from anastomoses
- Abdominal problems after eating such as digesting
- Difficulties in emptying stomach
- Anorexia
- Diabetes

In pancreatic cancers located in pancreatic tail or body a distal pancreatectomy is advised. Additionally, spleen is often removed. (66)

Total pancreatectomy is performed in pancreatic carcinomas which require the removal of the whole pancreas. During this surgery the gall bladder, the spleen and a part of the stomach is removed. (66) Consequences of total pancreatectomy are insulin-dependent diabetes and digestive problems. (66)

1.10.1.8 Stent placement

In palliation the most frequent symptom requiring invasive treatment is presented by obstructive jaundice. As trials have shown that operative bypass procedure leads to more complications such as cholangitis or bile leak, stenting is the preferred technique. (68) However, stenting bears its own specific risks. Stent occlusion is a possible complication as well as acute cholangitis, although it is less common when using metal stents. (68)

Placement of stents is usually performed during endoscopic retrograde cholangiopancreaticography.

1.10.1.9 Bypass surgery

Although, as stated above, a bypass surgery may bear a higher rate of complications than stent placement, it also has some advantages. Thus, it is usually sustained for longer periods before further treatment is required and it is possible to apply local treatments during the surgery as local pain treatment. (66)

1.10.2 Locally resectable pancreatic cancer

The only curative therapy for pancreas cancer is the radical resection of the cancer with a R0 resection edge. R0 resection is defined that as removal the entire cancerous tissue with no visible or microscopic signs of cancer left behind. (69)

Within 6 weeks after surgery an adjuvant therapy for six months should be started:

- Modified FOLFIRNINOX
    - Fluorouracil
    - Leucovorin
    - Irinotecan
    - Oxaliplatin
- Gemcitabine monotherapy
- 5-Fluorouracil
- Gemcitabine and Capecitabine
- S-1

mFOLFIRINOX was presented at ASCO 2018 and presents the recommended first line treatment. In a randomized phase III study of first line-treatment patients who were treated with mFOLFIRNOX showed a significantly increased median disease free survival and a longer overall survival compared to those who were treated with gemcitabine. Considering the high toxicity due to mFOLFIRINOX, this therapy should be only offered to young and

fit patients with ECOG 0-1. In this study mFOLFIRINOX was associated with a disease free survival time of 21.6 months (95% CI 17.5-26.7) vs. 12.8months (95% CI 11.7-15.2), the median overall survival was 30.4months (95% CI 41.5- --) vs. 34.8 months (95 % CI 28.6- 43.8), the metastases free survival was 30.4months (CI 95% 21.6- --) vs. 17.7months (95% CI 14.2-21.7).(70) However, the rate of grade 3-4 adverse events amounted to 75.5percents and was higher in patients with mFOLFIRINOX than with gemcitabine, where these amounted to 51.1percent. (70)

The use of chemoradiotherapy is still controversial. No significant benefit of survival was shown in an European Organisation for Research and Treatment (EORTC) trial in addition of adjuvant chemoradiotherapy to chemotherapy with gemcitabine. (1) Thus ESMO guidelines 2015 suggest that adjuvant chemoradiotherapy should only be done in clinical trials. (1)

1.10.3 Locally Advanced Pancreatic Cancer (LAPC)

During the last decades no consistent recommendation has been established how to treat pancreatic cancers that are not resectable anymore but have not metastasized. The evaluation of resectability is presently guided by the encasement of the superior mesenteric artery and the superior mesenteric-portal vein such as the hepatic and the celiac artery by the tumour. (71) Locally advanced pancreatic cancer is divided into an A and B category, starting with an encasement >180° in arteries and an occlusion of superior mesenteric-portal vein without an obvious possibility for reconstruction. Borderline resectable pancreatic cancers have been defined by an abutment ≤180° of superior mesenteric artery and celiac artery, a short-segment abutment or encasement of hepatic artery without extension in the previous blood vessels or >50% narrowing of superior mesenteric-portal vein, portal vein or superior mesenteric vein with the possibility for reconstruction.
It seems reasonable to try to attain resectability of the tumor. ASCO 2016 guidelines suggest that, depending on baseline performance status, psychical status, ECOG performance status and comorbidities an initial systemic chemotherapy for 6 months should be provided. (72) As mentioned above, there is no general treatment recommendation due to the lack of randomized controlled trials. A radiation therapy or stereotactic body radiotherapy should be offered to patients with an ECOG performance

status ≤ 2 and a local progression without metastases after induction chemotherapy. (72) In stable disease with severe side effects due to chemotherapy radiation therapy may be an option. (72) Progression in pancreatic cancers should be treated as metastatic pancreatic cancers. (72)

There is evidence that patients developing suffering from resectable and borderline resectable pancreatic cancers may benefit from neoadjuvant therapy. (73) (74) Additionally, it is suggested that neoadjuvant treatment might increase the rate of R0 resections and may lower tumour stages. (74) (75)

1.10.4 Unresectable pancreatic cancer

1.10.4.1 First line

As the ASCO 2018 guidelines recommend, mFOLFIRINOX is the first line treatment in palliative setting as well. However, only patients with ECOG PS 0-1, at good health condition with the access to proper services for port and chemotherapy and a good management of the aggressive treatment should get this combination. (76)Alternatively, gemcitabine mono or in combination with nab-paclitaxel is also recommended as first line in the palliative setting. (76) Gemcitabine plus nab-paclitaxel is recommend in patients with ECOG PS 0-1 in a relatively good health condition, whereas Gemcitabine mono is preferred in patients with ECOG PS 2 or a comorbidity profile that does not allow an aggressive therapy treatment. (76) An option in this situation may be the combination with erlotinib or capecitabine. (76)

According to ASCO 2018 guidelines any anticancer treatment in patients with ECOG PS more than 3 or with poorly controllable comorbidities therapy should be individually decided. (76) Close attention should be on management of symptoms, supportive treatment and quality of life.

1.10.4.2 Second line

In second line treatment routine testing for MSI-H and dMMR should be done. The PD-1immune checkpoint inhibitor pembrolizumab is recommended for patients who are suitable and are dMMR or MSI-H positive. (76) Gemicitabine plus nab-paclitaxel appear a valuable option after first line treatment with FOLFIRINOX with an ECOG PS 0-1 and at a relatively good health condition, although it is not a strong evidence supporting this. (76) Fluorouracil in combination with nanoliposomal irinotecan, or fluoruracil plus irinotecan, is a possible treatment after first-line treatment with gemcitabine plus NAB-paclitaxel in patients with ECOG PS of 0 to 1 and at good health condition. (76) Fluorouracil in combination with oxaliplatin may be administered after first-line treatment with gemcitabine plus nab-paclitaxel in patients with ECOG PS of 0 to 1 with a relatively good health condition. (76)

There are not recommendations in the ASCO 2018 guidelines for third-line or further treatment in palliative setting.

1.11 Prognosis

It occurs that surgery is the only possibility to cure pancreatic cancer. Therefore, diagnostic in early stages of cancer is obviously advantageous. However, 80-85 percent of pancreatic carcinomas are in an advanced unresectable stadium at time of diagnosis. (40)

The 5-year survival rate is reported between 9 and 10 %. (16)(15)

1.12 Laboratory values

1.12.1 AST

AST is the abbreviation of aspartate-amino transferase, also called glutamic oxaloacetic transaminase (GOT). It transfers an amino group between aspartate and glutamate and in the course of this oxaloacetate is formed. (77) There exist two isoenzymes of this protein in

humans. . One isoform is located in the mitochondria and referred to as m-AST and the other isoform is present in the cytoplasm as c-AST. Both isoenzyms are mainly present in liver tissues, myocardium and striated muscle. (78) An elevated level of AST in blood serum is an indication of cell damage, particularly as a consequence of liver necroses or myocardial infarction. (78) 30 percent of AST is located in the cytoplasm and 70 percent in the mitochondria. (79) Nearly 80 percent of total AST in the liver is mAST. (80)

The AST plays an essential role in the mitochondria because it is a part of the malate-aspartate shuttle which is important to transfer NADH through the inner mitochondrial membrane from the cytosol so that it is provided as a substrate for the respiratory chain. Furthermore NAD+ is regenerated for glycolysis in the cytosolic compartment. In the cytosol, the malate dehydrogenase transfers two electrones from NADH to oxaloacete which is thereby reduced to malate. Malate is then transported to the mitochondrial matrix in exchange for α-ketoglutarate. In the matrix malate is again reconverted into oxaloactete by the malate dehydrogenase to build NADH for the respiratory chain. The AST transforms oxaloacete to aspartate and glutamate to α-ketoglutarate and thereby drives the reactions at the malate dehydrogenase enzymes. In addition, mitochondrial aspartate is exchanged for cytosolic glutamate making the substrates available again for AST activity. (77)

The half life of AST is 18 h, its degradation products are removed by the liver sinusoids. (77) (81)

1.12.2 ALT

ALT is the abbreviation of alanine-amino transferase which transfers amino groups between alanine and glutamate. In this reaction pyruvate is formed. (77) This process plays an important role in the anaerobic glycolysis.

Pyruvate is routinely formed by glycolysis. In parallel to anaerobic glycolysis, muscle proteins may be degraded to amino acids such as glutamate. The amino group carbon is then transferred to pyruvate by the activity of the ALT so that alanine and also α-ketoglutarate is built. Alanine is carried to the liver where it is transformed to pyruvate again by the ALT. Pyruvate may be used to produce glucose. (77) The ALT is located

only in the hepatocyte cytoplasm. (79)(80) ALT is removed by the liver sinusoids, the half time of the enzyme amounting to 36 h. (81) (77)

1.12.3 Gamma glutamyl transferase

Gamma glutamyl transferase, GGT, is an enzyme that plays an important role in the defense against reactive oxygen species. Gamma glutamyl transferase catalyzes the donation of a gamma glutamyl group from glutathione to the amino group of another amino acid as to build cystine. (82) (83) GGT is usually expressed on the luminal surface of exocrine cells in ducts and glands with high concentration in the renal tubule cells and hepatic bile canaliculi. (84) GGT is also expressed in other tissues such as in the sweat glands, prostatic acini, testicular tubules and intestinal crypts. (84) It is supposed that GGT divides amino acids from extracellular glutathione and provides amino acids for intracellular glutathione synthesis. (85) (86) As glutathione is important for cells exposed to oxidative stress, GGT provides the essential amino acid cystine for building intracellular glutathione.
Levels of serum gamma glutamyl transferase may be elevated due to slight cholestasis, cell damage or chronic alcohol consumption. (87)

1.12.4 Alkaline phosphatase

Alkaline phosphatase is the description for enzymes that hydrolyse phosphoric esters. (88) As these are located in liver and gallbladder tissue as well as in bone mass, elevated alkaline phosphatase levels in serum reflect diseases relating to these tissues. (87) Furthermore, increased GGT levels are seen in pregnancy. (87)

1.12.5 Cholinesterase

Cholinesterase, particularly butyrylcholinesterase, is an enzyme produced by hepatic cells to divide choline esters. Butyrylcholinesterase, also called “non specific cholinesterase” or pseudocholinesterase, is related to the acetylcholinesterase which plays an important role

in neuronal transmission. (89) Butyrylcholinesterase is essential to terminate the function of succinylocholin. (89) As cholinesterase reflects liver function it is reduced in liver cirrhosis. (90) There are hints that a decreased butyrylcholinesterase activity may negatively correlate with systemic inflammation. (91)

1.12.6 Prothrombin time

The parameter referred to as prothrombin time is an indicator of the exogen pathway of coagulation measured in citrate blood. Prothrombin time thus reflects the function and presence of vitamin K-dependent coagulation factors II, VII, IX and X which are synthesized in the liver. (92) Vitamin K is the cofactor in carboxylation of these coagulation factors. Although, prothrombin time is a value to measure coagulation, in liver cirrhosis it does not reflect the bleeding risk. (92)

1.12.7 De Ritis-Ratio

The De-Ritis ratio is the ratio between AST and ALT and was first described by Fernando De Ritis in 1957. (93) These two transferases play an important role in the urea cycle because of their function as transporters of nitrogen to the liver. As a result amino acids are transformed to keto acids by transferring amino groups. An important coenzyme in this reaction seems to be pyridoxyl-phosphate. The reaction may proceed in both directions. α-ketoglutarate is known as the keto acid for both AST and ALT. (77)

1.12.7.1 De-Ritis Ratio as clinical laboratory parameter

Slight damages of liver cells cause a higher elevation of ALT than AST. Accordingly, the De-Ritis ratio is lower than 1. Herold et al consider that in heavy liver cell damages the AST/ALT ratio is higher than 1. (79) There is evidence that a De-Ritis ratio over 1 is associated with hepatocyte apoptosis. (94) Considering that the AST is increasing due to damage of myocardial cells and striated muscles other laboratory parameters such as lactate dehydrogenase and total creatine kinase should be taken in consideration as important additional laboratory values in the diagnostic of myocardial infarction. (78) An

elevated De-Ritis ratio indicates for instance an acute viral hepatits with poorer prognosies. (93) (77) Liver metastases and chronic damage due to alcohol lead to an increased AST/ALT ratio, as well. (87)

2 Materials and Methods

We retrospectively evaluated pre-diagnostic clinicopathological data on 528 patients with adenocarcinoma of the pancreas, treated between 2005 and 2014. The potential prognostic value of AST, ALT, GGT, bilirubin, ALP, prothrombin time ands CHE was analyzed using correlation analysis with the Kaplan-Meier method, and univariate and multivariate Cox proportional regression models.

2.1 Patients

Patient who were diagnosed with pancreatic cancer or treated between 2005 and 2014 at the oncology Graz or in external hospitals of the KAGES were included in this study. Laboratory values before date of diagnoses, staging and information about survival status were documented. Both clinic-pathological and pathological data was retrieved from clinical records at the Division of Clinical Oncology and from the Institute of Pathology.

2.1.1 Date of diagnosis

For date of diagnosis we took the date of histological conformation. The histological samples were collected by pancreatectomy, endoscopic retrograde cholangiography, fine-needle aspiration biopsy or biopsy of liver tissue. In cases without any histological sample but strong clinic evidence confirmed by obduction we took date of clinic diagnosis.

2.1.2 Staging

Due to the fact that TNM classification changed we standardized staging of our study cohort according to the 7th edition. (95)

2.2 Laboratory values

We collected several laboratory values, such as alanine-aminotransferase, aspartate-aminotransferase, cholinesterase, alkaline phosphatase, bilirubin, gamma-glutamyl transferase, thromboplastin time, uric acid, albumin, Ca 19-9, c-reactive protein, fibrinogen, number of thrombocytes, leucocytes, neutrophils, lymphocytes, monocytes, basophils and eosinophils from a blood sample within 7 days up to two weeks before date of diagnosis. For our analysis we took laboratory values from routine blood drawings.

2.2.1 Standards of laboratory values:

Bilirubin 0.10-1.2 mg/dL
Alkaline phosphatase 40-130 U/L
Gamma-glutamyl transferase -55 U/L
Alanine aminotransferase -35 U/L
Aspartate aminotransferase -45 U/L
Cholinesterase 3900- 11000 U/L
Prothrombin time 70-120%

2.2.2 Follow- up period

Within the first 3 years follow-up evaluation was performed every 3 months, followed by biannual evaluations for 5 years. After this period annual follow ups were performed in patients with curative resected tumour stages. Date of death in deceased patients were collected from data in the central registry of the Austrian Bureau of Statistics or phone calls to their relatives.

2.3 Ethics statement

Prior to any patient-related acitivites our retrospective database study was approved by the local ethics committee (Ethics Committee of the Medical University of Graz, Austria; document number No. 26-196 ex 13/14). Based on the granted "waiver of consent" no written informed conset was requested from individual patients. We took care that all investigation were performed strictly adhering to the declaration of Helsinki principles.

2.4 Statistical analyses

Cancer-specific survival was determined as the time (given in months) from date of histologically confirmed diagnosis or surgery to cancer-related death. Non-parametric tests as the Mann-Whitney U and χ^2test were used to calculate the association between bilirubin, AST, ALT, GGT, ALP, CHE and prothrombin time and clinic-pathological parameters.

For identification of the optimal cut-off levels for the abovementioned laboratory values we used receiver operating curve (ROC) analysis by MedCalc (Windows version 18.5, MedCalc Software bvba, Ostend, Belgium). (96)(97)

To calculate patients´ clinic endpoint we utilized the Kaplan-Meier method. Multivariate Cox proportion analysis was performed to identify independent prognostic parameters. We reported Hazard ratios (HRs) as relative risk with corresponding 95% confidence interval (CIs) from Cox analysis. Multivariate Cox analysis and univariate analysis were performed utilizing Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). We considered a two-sided *p-value* <0.05 statistically significant.

3 Results

Our cohort included 574 patients, 268 females and 396 males, with pancreatic cancer. 143 patients, i.e. 15 % of these, had stage I or II-pancreas cancer at time of diagnosis, 31 (5.4%) were diagnosed with stage III, and the largest population with 399 patients, 69.6 %, with stage IV. The tumour grading was in 343 of pancreatic cancers G I or II, which presented 59.8 %. 231 patients, which is 40.2 %, had a tumour grading G III or IV. Chemotherapy was given to 410 patients, 71.6 % and a surgical resection was performed in 173 patients, 30.1%. These data are summarized in Table 3.

Table 3. Characteristics of study cohort

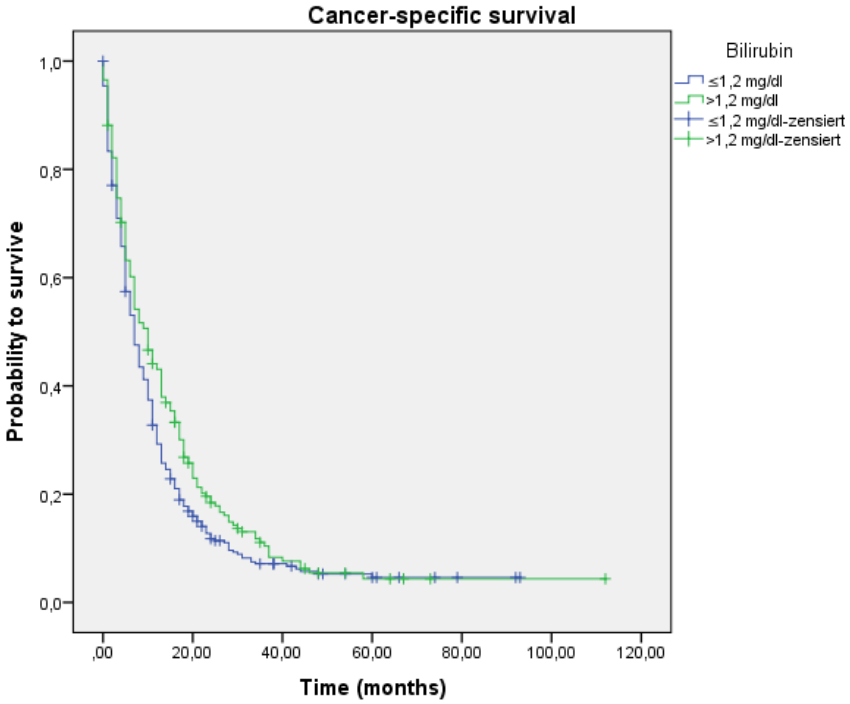
Characteristics		Number	Percent
Gender			
	Female	268	46.7
	Male	306	53.3
Tumour stage			
	I + II	143	25
	III	31	5.4
	IV	399	69.6
Tumour grade			
	I + II	343	59.8
	III + IV	231	40.2
surgical rescection			
	Yes	173	30.1
	No	401	69.9
Chemotherapy			
	Yes	410	71.6
	No	163	28.4
Karfnosky Index			
	missing cases	5	9
	<80	337	58.7
	90-100	232	40.4
Cancer specific survival			
	Alive	53	9.2
	Dead	521	90.8

The median survival time was 7 months (range 0-112 months). The median of pre-treatment total serum bilirubin was 0.9 mg/dL, the median of gamma- glutamyl transferase was 157.50 U/L, the median of aspartate-amino transferase was 34 U/L, the median of

alanine-amino transferase 45 U/L, the median of alkaline phosphatase 145 U/L, the median of cholinesterase was 6565 U/L and the median of prothrombin time was 98%. In our cohort the median of Ca 19-9 was 809.5U/L.

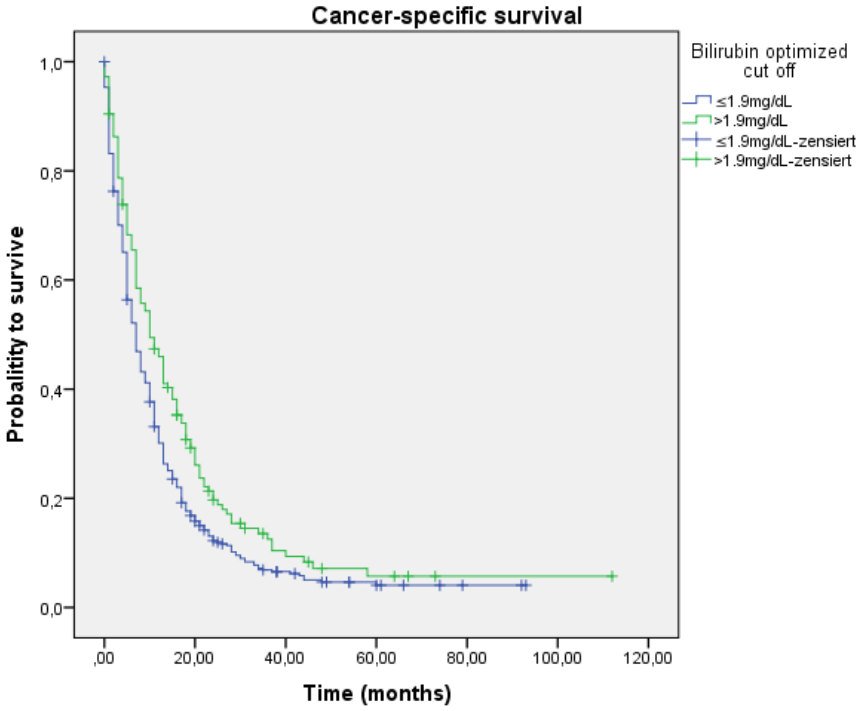
As noted before, the median total serum bilirubin was 0.9mg/dL. For Kaplan Meier analysis we formed two groups in association with the used upper limit value which is 1.2mg/dL in our clinic. A number of 349 patients had a total serum bilirubin ≤ 1.2mg/dL and 202 patients had total serum bilirubin levels >1.2mg/dL. Total serum bilirubin levels were significantly associated with a longer cancer-specific survival (CSS) (p-value 0.25). The median cancer specific survival in patients with ≤1.2mg/dL was 7 months (%95 CI 5.87 to 8.13) versus 10 months (95% CI 7.85 to 12.15) in patients with >1.2mg/dL. (Figure 1)

Figure 1. Kaplan Meier Analysis with total serum bilirubin ≤1,2mg/dL



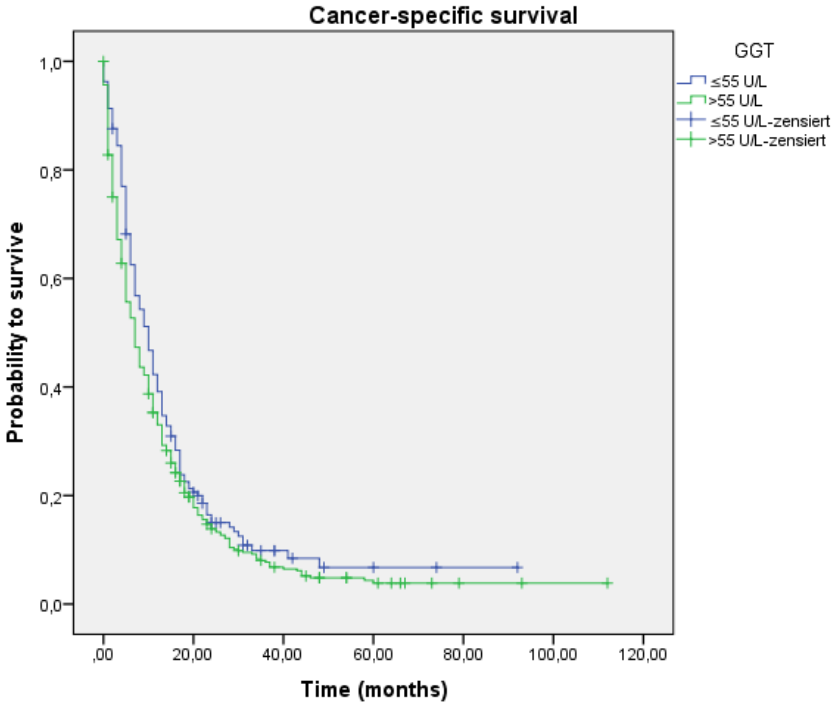
The calculated optimized cut off with ≤1.9mg/dL significantly associated with longer cancer-specific survival with a median of 10 months (95% CI 7.23 to 12.77) in the cohort with a total serum bilirubin of >1.9mg/dL vs. a median of 7 months (95 %CI 5.69 to 8.12) with a total serum bilirubin of ≤1.9mg/dL (p-value= 0.003). (Figure 2)

Figure 2. Kaplan Meier Analysis with total serum bilirubin ≤1,9mg/dL



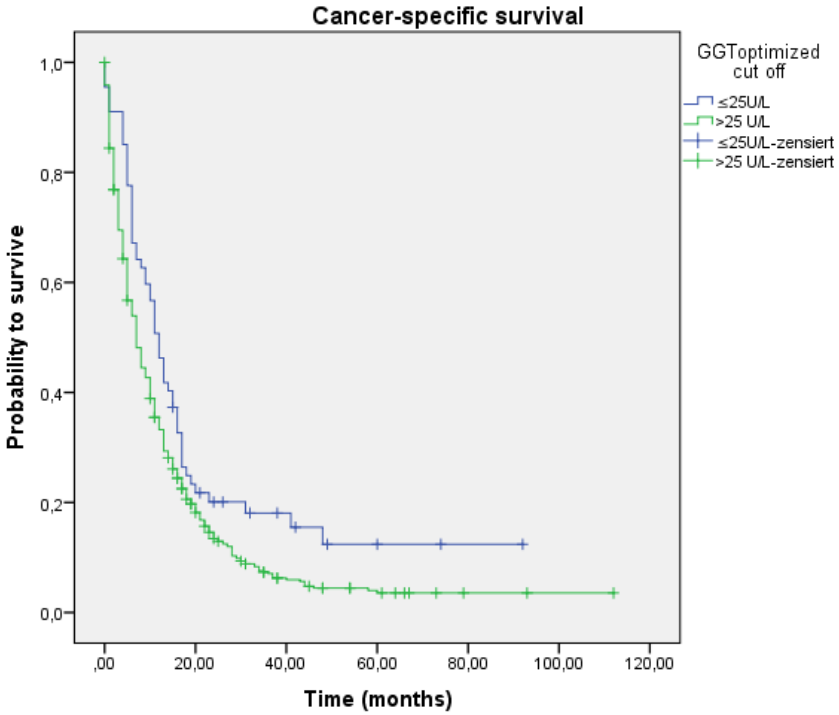
There was no significant association in gamma glutamyl transferase levels with cancer-specific survival. However, a pretreatment GGT ≤55 U/L was associated with a median CSS of 10 months (95% CI 7.87 – 12.14) vs. 7 months (95%CI 5.79 - 8.21) in patients with levels >55 U/L (p-value =0.062). (Figure 3)

Figure 3. Kaplan Meier Analysis with GGT ≤55U/L



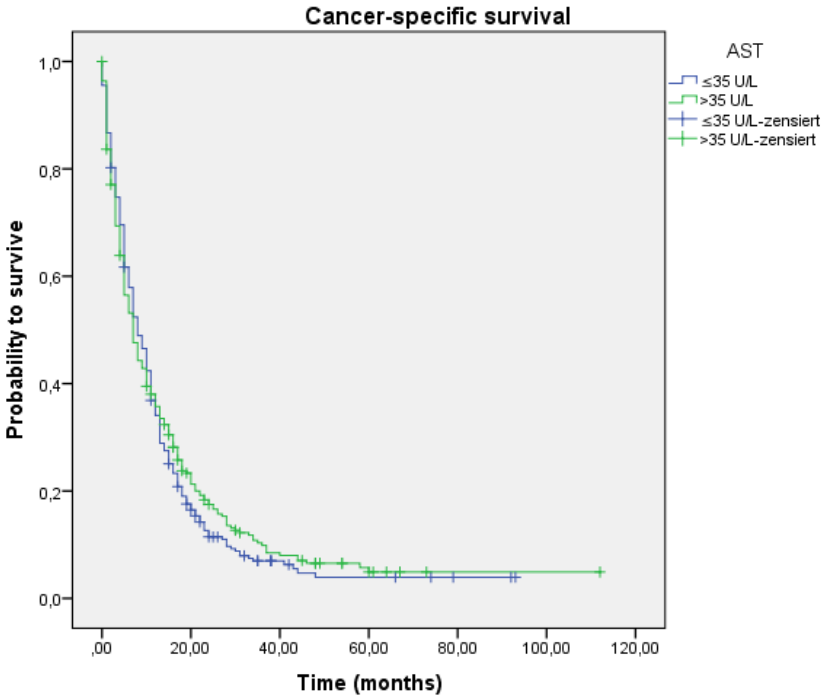
A GGT ≤25U/L was associated with longer CSS with a median of 12 months (95%CI 9.6 - 14.4) vs. 7 months (95% CI 5.93 – 8.07) (p-value=0.007). (Figure 4)

Figure 4. . Kaplan Meier Analysis with GGT ≤25U/L



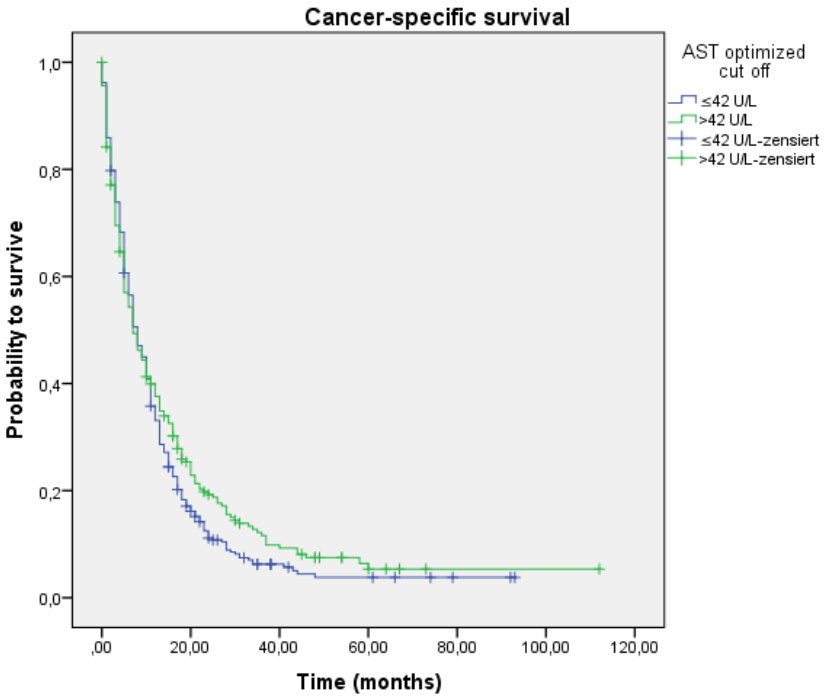
Serum levels of an aspartate aminotransferase ≤35 U/L were not significantly associated with longer cancer-specific survival with a median of 8 months (95%CI 6.52 – 9.48) vs. 7 months (95% CI 5.54 – 8.46) (p-value = 0.509). (Figure 5)

Figure 5. Kaplan Meier Analysis with AST ≤35U/L



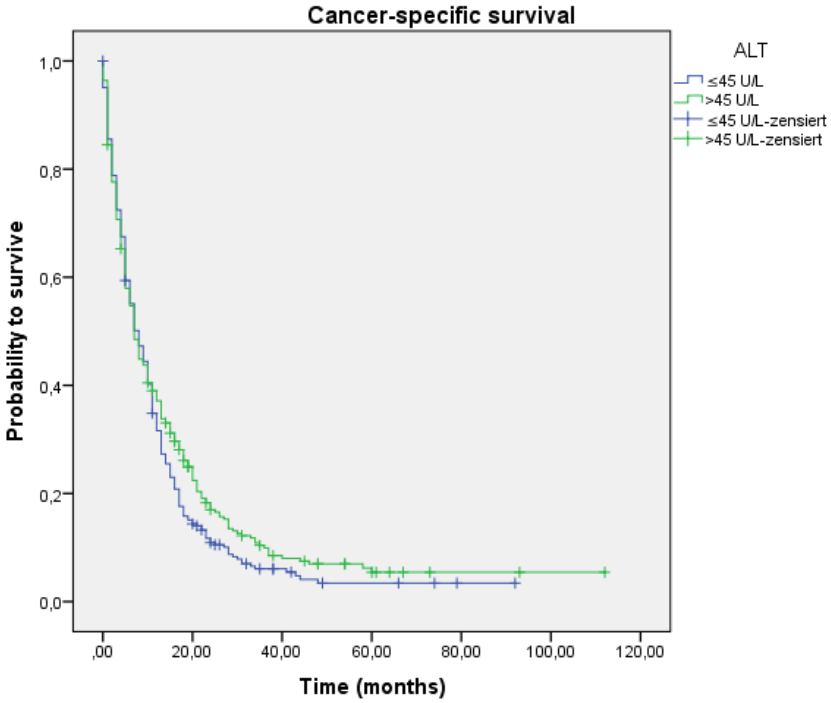
Furthermore, serum AST values lower than the optimized cut off of ≤42 U/L did not significantly influence cancer specific survival with a median of 8 months (95% CI 6.62 – 9.38) vs. 7 months (95% CI 4.92 – 9.08) (p-value = 0.144) (Figure 6)

Figure 6. Kaplan Meier Analysis with AST ≤42U/L



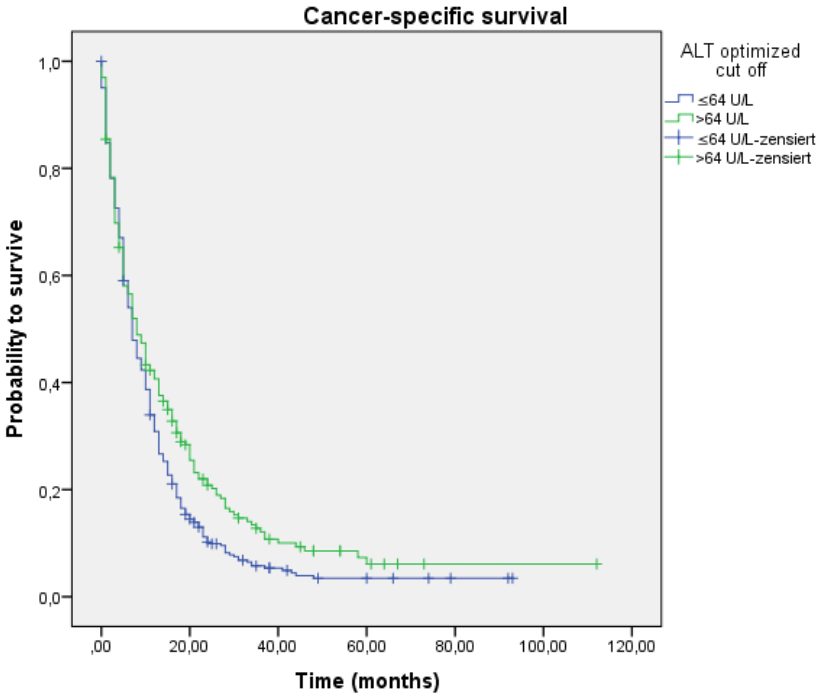
Our analysis showed no significant correlation of elevated serum alanine transaminase values used in clinical practice with cancer specific survival. The median was 8 months (95% CI 6.36 – 9.64) in patients with pre treatment ALT levels ≤45 U/L vs. 7 months (95% CI 5.65 – 8.35) in patients with ALT levels >45 U/L (p-value = 0.107). (Figure 7)

Figure 7. Kaplan Meier Analysis with ALT ≤45U/L



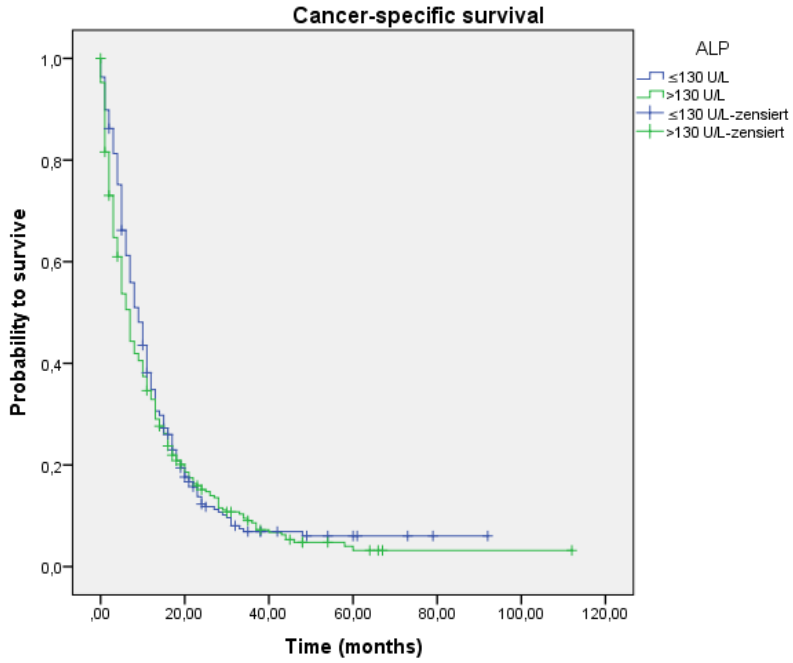
In spite of previous data, our optimized cut off of ≤64 U/L showed a significant relation with cancer specific survival. Interestingly, serum ALT values ≤64 U/L were correlated with a decreased cancer specific survival with a median of 7 months (5.93 – 8.07) compared to a median of 8 months for ALT values >64U/L (95% CI 5.89 – 10.11) (p-value =0.009) (Figure 8)

Figure 8. Kaplan Meier Analysis with ALT ≤64U/L



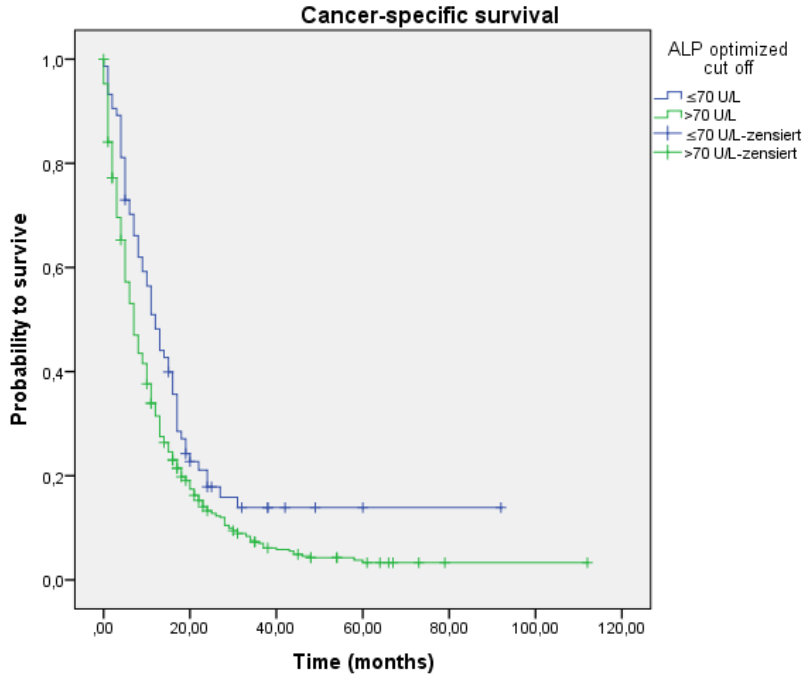
Alkaline phosphatase (ALP) values ≤130 correlated with longer cancer specific survival with a median of 9 months (95%CI 7.48 – 10.53) vs. a median of 7 months for values >130U/L (95% CI 5.97 – 8.03). The difference was not significant, though (p-value = 0.206) (Figure 9)

Figure 9. Kaplan Meier Analysis with ALP ≤130U/L



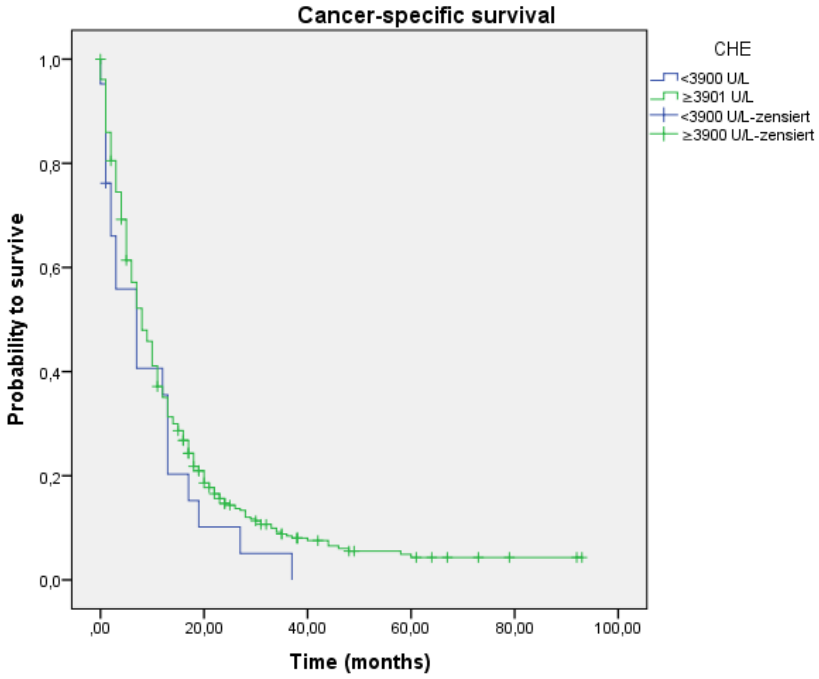
Our data showed a significant longer CSS with a median of 12 months (95% CI 9.23 – 14.77) in patients with ALP values ≤70 U/L vs. 7 months in patients with ALP values >70U/L (95% CI 6.00 to 7.8) (p-value= 0.005). (Figure 10)

Figure 10. Kaplan Meier Analysis with ALP ≤70U/L



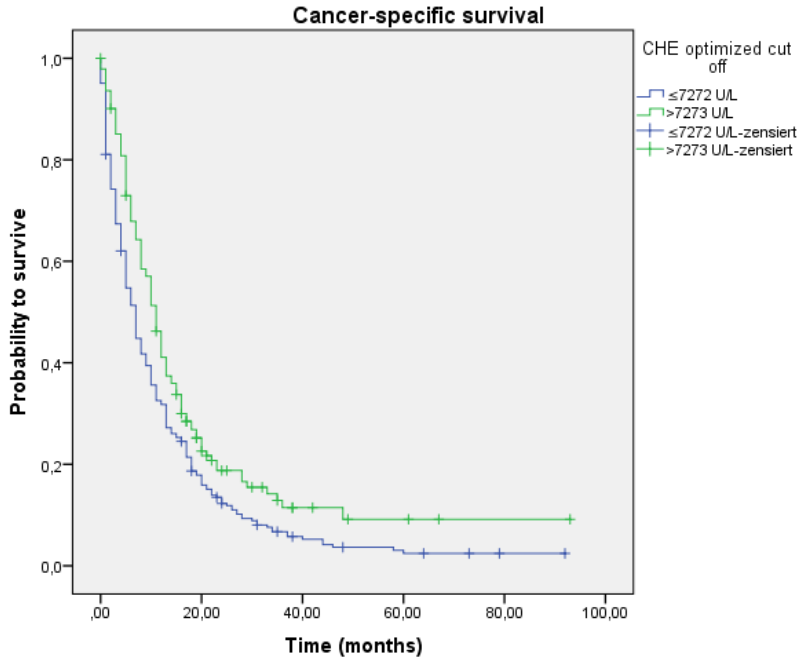
Pre-treatment cholinesterase values of <3900 U/L were not significantly associated with shorter cancer-specific survival. The median was 7 months (95%CI 1.34 – 12.66) vs. 8 months (95% CI 6.75 - 9.25) with values ≥3900 U/L (p-value = 0.216) (Figure 11)

Figure 11. Kaplan Meier Analysis with CHE <3900U/L



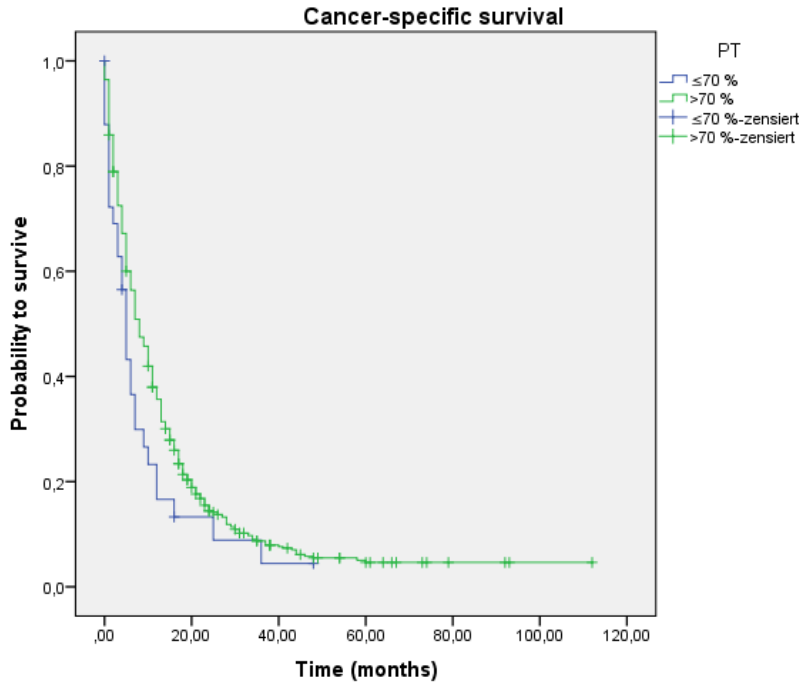
Interestingly, calculated with the optimized cut off of ≤7272 U/L the association was significant. The median was 11 months (95% CI 5.95 – 8.05) in the group with serum CHE values >7272 U/L vs. 7 months (95% CI 5.95 – 8.05) (p-value= 0.001). (Figure 12)

Figure 12. Kaplan Meier Analysis with CHE ≤7272 U /L



A prothrombin time ≤70% was associated with a shorter cancer specific survival of 5 months (95% CI 3.63 – 6.31) vs. 8 months (95% CI 6.82 – 9.18), although, this association was not significant (p= 0.057). (Figure 13)

Figure 13. Kaplan Meier Analysis with PT ≤70%



Univariate and multivariate cox analyses were performed to investigate the association of clinical outcome with the abovementioned laboratory values.

In univariate cox analysis higher grading, higher staging, no chemotherapy and surgical resection done, elevated Ca 19-9, total serum bilirubin levels ≤1.2mg/dL and ≤1.9mg/dL, GGT levels >25U/L, ALT levels ≤64 U/L, ALP levels >70U/L and CHE levels ≤7272U/L were significantly associated with a shorter cancer-specific survival. (Table 4)

In multivariate cox analysis chemotherapy, Ca19-9, staging, grading bilirubin with the optimized cut off 1.9mg/dL and CHE with the optimized cut off 7272 U/L were significantly associated with cancer specific survival. (Table 4)

Table 4. Univariate and multivariate Cox proportinal analysis regarding cancer-specific survival in pancreatic cancer patients

	univariate analysis		multivariate analysis	
parameter	Hazard ratio	p-value	Hazard ratio	p-value
Gender				
male	1 (reference)		1 (reference)	
Female	1.159 (0.975 - 1.377)	0.94	1.003 (0.794 - 1.266)	0.98
Grading				
G1 + G2	1 (reference)		1 (reference)	
G3 + G4	1.269 (1.065 - 1.512)	0.008	1.699 (1.342 - 2.15)	<0.001
Staging				
Stage I + II	1 (reference)		1 (reference)	
Stage III	3.161 (2.099 - 4.761)	<0.001	2.254 (1.367 - 3.717)	0.001
Stage IV	3.789 (2.995 - 4.794)	<0.001	3.001 (2.178 - 4.136)	<0.001
Chemotherapy				
No	1 (reference)		1 (reference)	
Yes	0.412 (0.339 - 0.501)	<0.001	0.329 (0.251 - 0.432)	<0.001
Surgical resection				
No	1 (reference)			
Yes	0.339 (0.775 - 0.418)	<0.001		
Ca 19-9				
≤1191.7 U/ml	1 (reference)		1 (reference)	
>1191.7 U/ml	1.872 (1.554 - 2.256)	<0.001	1.288 (1.015 - 1.635)	0.037
Bilirubin				
≤1.2mg/dL	1 (reference)			
>1.2mg/dL	0.819 (0.682 - 0.983)	0.032		

≤1.9mg/dL	1 (reference)		1 (reference)	
>1.9mg/dL	0.746 (0.610-0-913)	0.004	0.694 (0.502 - 0.96)	0.027
GGT				
≤55U/L	1 (reference)			
>55U/L	1.193 (0.983 - 1.447)	0.073		
≤25U/L	1 (reference)		1 (reference)	
>25U/L	1.443 (1.093-1.905)	0.010	1.1 (0.717- 1.686)	0.663
AST				
≤35U/L	1 (reference)			
>35 U/L	0.945 (0.795 - 1.124)	0.526		
≤42U/L	1 (reference)		1 (reference)	
>42U/L	0.880 (0.737 - 1.052)	0.160	1.017 (0.713 - 1.45)	0.925
ALT				
≤45U/L	1 (reference)			
>45U/L	0.872 (0.733 - 1.038)	0.122		
≤64U/L	1 (reference)		1 (reference)	
>64U/L	0.791 (0.658 - 0.951)	0.013	0.876 (0.623 - 1.231)	0.446
ALP				
≤130U/L	1 (reference)			
>130U/L	1.117 (0.934 - 1.335)	0.225		
≤70U/L	1 (reference)		1 (reference)	
>70U/L	1.440 (1.101 - 1.884)	0.008	1.406 (0.937 -2.11)	0.1
CHE				
<3900 U/L	1 (reference)			
≥3900U/L	0.761 (0.485 - 1.198)	0.237		
≤7272U/L	1 (reference)		1 (reference)	
>7272U/L	0.711 (0.570 - 0.885)	0.002	0.706 (0.55 - 0.907)	0.006
PZ				
≤70%	1 (reference)		1 (reference)	
>70%	0.706 (0.485 - 1.028)	0.069	0.777 (0.466 - 1.293)	0.331

4 Discussion

Prognostic markers play an essential role in decision-making processes for the most appropriate therapy. However, at present the diagnosis of pancreatic cancer is associated with a very poor prognosis. Therefore it is critically important to evaluate affected patients with regard to their compatibility for specific treatments. FOLFIRINOX is the current state of the art in treatment of pancreatic cancer, but in spite of the significant longer overall and cancer free survival, it involves a high risk for comorbidities. (70) In consequence, a well-founded and patient-specific evaluation of the application of this highly toxic therapy appears to be essential.

Overall, in our study we investigated general clinic laboratory parameters used in daily routine as diagnostic and follow-up parameters in the investigation of various diseases. In a univariate analysis of the laboratory data of 574 patients diagnosed with pancreatic cancer elevated serum bilirubin levels, lower levels of GGT, lower levels of ALT, lower alkaline phosphatase levels, and higher cholinesterase levels were found to be significantly associated with a better prognosis. It must be stated, however, that for most parameters significance was obtained using optimized cut off values except for bilirubin which was significant using the clinical upper limit. In multivariate analysis only bilirubin and serum cholinesterase could be determined as independent prognostic markers.
In a Kaplan Meier analysis calculated with the optimized cut off levels of bilirubin, GGT, ALT, alkaline phosphatase and cholinesterase a significant association between these parameters and cancer specific survival was observed.
This may not be too surprising given that these parameters in particular reflect the health condition and function of the liver.

As to the mechanistic links between these parameters and their prognostic value we can at present only speculate. For example, in the case of gamma-glutamyl transferase it should be considered that this enzyme has been found to be altered in several cancer types and has been reported to play a role in the resistance to chemotherapy as it provides cysteine for intracellular synthesis of glutathione which protects cells from oxidative stress induced by therapeutic drugs. (98)(85) Considering that GGT is usually highly expressed in pancreatic and liver tissue it may not be regarded a useful prognostic marker in cancerous lesions in these organs. However, studies have shown an association of pretreatment GGT levels with

a poorer outcome in hepatocellular carcinoma. (99) (100) In addition, elevated pre-therapeutic GGT levels were also identified as markers for poor prognosis in other cancer types such as for instance ovarian cancer, breast cancer and esophageal squamous cell cancer. (101) (102) (103)(103) (104)
In a previous study evaluating prognostic markers in unresectable pancreatic carcinomas Engelken et al have already shown an association of GGT with survival, both when applying univariate as well as multivariate analysis. (105)

Values of alkaline phophatase >70U/L were associated with a shorter cancer specific survival in our study. These results could be obtained in univariate analysis and with Kaplan Meier analysis. In comparison, when we performed multivariate analysis alkaline phosphatase could not be established as an independent prognostic marker. In contrast, Hashimoto et al. have shown a significant association in multivariate analysis of alkaline phosphatase values > 300 U/L with overall survival. (106) This may be due to the facts that the cut off used in their study was higher than the cut off value used in the present one and that only recurrent or metastatic patients were included by Hashimoto and colleagues. Furthermore, the primary end point evaluated was overall survival.

In an analysis of two different phase III studies by Stocken et al., albumin, LDH and white blood cells were defined as highly significant independent prognostic markers. Furthermore, the presence of metastases, alkaline phosphatase, bilirubin and AST and additionally BUN (blood urea nitrogen) were supposed to serve as potential prognostic markers. (107) By univariate cox analysis an association of AST, alkaline phosphatase, albumin, LDH, WBC, metastases and bilirubin to survival rate was shown. (107) BUN and tumour stage were no significant prognostic markers. (107)
Again, methodological differences may explain some of the discrepancies with the present investigation. Thus, the study by Stocken and colleagues evaluated reference values which are used in the clinical practice and were randomized 15 and 20 days after diagnosis, and the primary outcome examined was the survival time defined as the time between the date of diagnosis and the date of death from any cause. (107) Interestingly, with a number of 653 patients included in their study the cohort size was similar to our cohort. Furthermore, disagreements between both studies may also be caused by the different statistic tools applied. Considering the different results regarding multivariate analysis of categorized

parameters as used in daily routine, there are still other discrepancies such as that our data did not reveal independent prognostic parameters in multivariate analysis categorized by clinically used cut offs. In addition, we used pre-diagnostic parameters in pancreatic cancer patients in all stages whereas Stocken et al. investigated laboratory values randomized 20 and 15 days after diagnosis only in patients with unresectable pancreatic cancer that were eligible for chemotherapy. This may give an explanation for the association of elevated bilirubin levels with better prognosis detected in this study. Moreover, bilirubin was associated with the location in the pancreatic head in our present study and this location correlated with a longer cancer specific survival due to its relation to an earlier stage of the cancer (Data not shown). This may confirm the fact that carcinomas located in the pancreatic head lead to an earlier detection and diagnosis since they already elicit detectable symptoms in the lower stages. However, GGT and ALP which besides bilirubin serve as cholestatic parameters revealed a positive association with a poorer outcome. Noteworthy, bilirubin in general is a rather variable laboratory value, making the predictive value of GGT and ALP the more valuable.
Finally, a potential cause for discrepancies is also the fact that the primary end point defined was the survival time between randomization and death from any cause.

Hashimoto et al. examined prognostic factors in 326 patients that received first line treatment with gemcitabine in an advanced stage of pancreatic cancer. Laboratory values collected at baseline were evaluated with respect to their association to time to treatment failure (defined as the time between the start of therapy and death or last follow-up). (108) Ca 19-9 was significantly elevated in the metastatic cohort compared to the recurrence cohort (108). Univariate cox analysis revealed recurrent and metastatic status as significant predictive factor (HR 0.53, p-value = 0.0002). (108) Other significant parameters detected were Karnofsky Performance status (HR 1.73), white blood cell count (HR 0.63), hemoglobin (HR 1.48), alkaline phosphatase (HR 0.49), aspartate aminotransferase (HR 0.69), alanine aminotransferase (HR 0.59), lactate dehydrogenase (HR 0.51), CRP (HR 0.40), and Ca 19-9 (HR 0.76) (108). Additional significant associations were found between the presence of liver metastases and peritoneal metastases to survival. (108) Independent prognostic factors conformed also in multivariate analysis were the Karnofsky performance status (HR 1.42, 95%CI 1.02 -2.01), alkaline phosphatase (HR 0.59, 95%CI 0.43 – 0.81), lactate dehydrogenase (HR 0.55, 95% CI 0.42 – 0.73) and CRP (HR 0.56, 95% CI 0.42 – 0.75). (108) Furthermore, the presence of liver and peritoneal metastasis proved to be independent prognostic markers. (108) Contradicting our present results,

alkaline phosphatase was identified as independent prognostic marker. However, Hashimoto et al. used higher cut off value of >330U/L, which may explain this difference. Moreover, the cohort examined was not similar to our population as they investigated patients with recurrence and metastatic pancreatic cancer eligible for chemotherapy.

Despite of the suggested role of AST and ALT in supporting tumour cell proliferation, these enzymes did not appear to be significant independent prognostic markers in our study. (109) (110) The results are in agreement with the findings of Huang et al. who performed a retrospective study regarding liver function tests in esophageal squamous cell carcinoma. (104) While there was no significant association of ALT and AST with overall survival in multivariate analysis, elevated ALT values did show such an association. (104) Huang et al. additionally evaluated the ratio between AST and ALT and found a significant association with overall survival in both univariate and multivariate analysis. (104)
Takayoshi et al. estimated AST as independent prognostic factor in multiple myeloma. (111) However, this trial included only 44 patients.
Considering that in our investigation higher values of ALT were associated with a longer cancer specific survival in univariate analysis, calculating the AST/ALT ratio would appear very interesting. Thus, although AST and ALT could not be identified as independent prognostic markers here, the quotient of enzyme activities which is also called "De Ritis ratio" may nonetheless present an independent prognostic marker.
In fact, the AST/ALT ratio turned out to be a significant prognostic marker not only in esophageal squamous cell carcinoma but also in various other cancer entities as for instance in upper tract urothelial cancer, gastric adenocarcinoma, head and neck cancer and renal cell carcinoma. (112) (113) (114) (115)

A multicenter, pooled analysis in Germany of two phase II trials identified pre-treatment bilirubin as independent prognostic marker besides Ca19-9 and CRP in locally advanced and metastatic pancreatic cancer. (116) Despite of the result that time to progression was not correlated with bilirubin levels in this study, overall survival was significantly associated with this parameter in univariate analysis (>1.0 mg/dL HR 1.62, 95% CI 1.18 – 2.24, p-value= 0.003; log [Bilirubin] HR 1.43, 95% CI 1.20 – 1.70, p-value <0.0001). (116) Nevertheless, bilirubin was not confirmed as independent prognostic marker by multivariate analysis. In comparison with our present study, there were some differences in

size and composition of the study population, sinceonly locally advanced or metastatic pancreatic cancer patients were included in the cohort comprising 291 patients.

Our current study revealed cholinesterase as a significant independent prognostic marker calculated with our optimized cut off value of < 7272 U/L. In contrast, there was no significance revealed with the cut off used in daily routine. High levels of serum cholinesterase reflect a good liver function. Considering that pancreatic cancer tends to metastasize to the liver it may be a marker for metastatic and systemic disease. Zivkovic et al. found a negative association between levels of butyrylcholinesterase and systemic inflammation (91). Suggesting that carcinomas provoke an inflammatory response, low levels of cholinesterase may thus be a sign of a systemic reaction. (117) However, at present this remains speculative and this hypothesis needs to be confirmed in future studies.

In a study with 75 pancreatic cancer patients at recurrence a correlation between low cholinesterase levels and histologically confirmed nerve plexus invasion was revealed. Low cholinesterase levels <300 IU/L were associated with cachexia, anemia, low albumin and ascites. However, there was no association with the presence of liver metastasis and only a slight association (p-value 0.065) with peritoneal dissemination detected. (118)

In an older study Kaniaris et al. investigated levels of serum cholinesterase in 146 healthy subjects in comparison to CHE levels in 180 cancer patients. (119) Despite of cholinesterase values ranging within the normal limits in cancer patients, they were on average significantly lower compared to healthy controls. The highest percentage of patients with low serum cholinesterase values was found in the cohort with hepatic metastases.

In line, low serum cholinesterase levels were associated with a poorer prognosis in our study, confirming the previous report. Thus, it would seem interesting to evaluate the relation of low serum cholinesterase values to the presence of liver metastases in our cohort.

Yanamoto et al. showed a significant correlation of cholinesterase to overall survival in 523 colorectal carcinoma patients. (120) The used cut off was the mean serum cholinesterase of >266 U/L which was identified as an independent prognostic marker (HR 0.995, 95% CI 0.992 – 0.998, p-value= 0.002). (120)

Differing from these studies, there is also an older investigation which showed lower but within the normal range cholinesterase levels in patients with cancer compared to healthy patients and thus suggested cholinesterase to be a poor prognostic factor. (121)

In spite of hypercoagulopathy being observed as paraneoplastic phenomenon, prothrombin time in our study was not identified as prognostic marker. Nonetheless, prothrombin time values >70 % tended to be associated with a better cancer specific survival (p-value= 0.069) in univariate analysis. Furthermore, in Kaplan Meier model a prothrombin time >70% was significantly associated with a 3 months longer survival than in patients with values of prothrombin time ≤70 %.
A study performed by Sun et al. reported higher levels of PT, INR, APTT, DD, F-VIII and fibrinogen and lower levels of PC and AT III in patients with pancreatic cancer compared to the control group. (122) In univariate analysis a significant correlation of INR, fibrinogen and D-dimerto overall survival was shown. D-dimer was the only independent prognostic marker in multivariate analysis. Prothrombin time showed a slight correlation with the presence of metastases (p-value= 0.03), and a significant correlation with INR, AT-III and D-dimer. Considering the strong significance of D-dimer as prognostic marker in this trial a comparison to our current study cohort would be interesting, specifically the evaluation of an association of INR with clinic-pathological parameters such as metastases, grading and staging.
A trial including 325 patients with unresectable pancreatic cancer by Engelken et al showed similar results as a longer prothrombin time was significantly associated to shorter survival in univariate analysis. However, in multivariate analysis c-reactive protein, leukocytosis and GGT apeared as independent prognostic markers. (105)

It must be mentioned that our study had some limitations, particularly due to the fact that we used pre-diagnostic markers. This might, e.g., explain the strong association of cholestatic parameters such as bilirubin with cancer-specific survival. More specifically, in our study high levels of bilirubin were associated with enhanced CSS which may reflect this circumstance. Moreover, bilirubin values may change quickly and their determination may thus produce highly variable results. Conducting chi-square analysis, an association of elevated total serum bilirubin with the location of pancreatic cancer in the pancreatic head was observed. In line, pancreatic cancers in stages I and II are more likely to be detected in the head of the pancreas. Cholestasis may thus lead to an earlier diagnosis.

Other increased cholestatic parameters such as alkaline phosphatase and gamma-glutamyl transferase were associated with a shorter cancer specific survival. In comparison with bilirubin, these two parameters tend to change slowly.

5 Conclusio

- Our study evaluated liver enzymes and liver synthesis parameters as prognostic markers for pancreatic cancer. Considering the variability of cut-off values evaluated in studies done before it would seem essential to perform further and more extended trials so as to estimate truly appropriate cut off, since cut-offs routinely applied in the clinical practice may not be suitable, as shown by our study.

- Low levels of cholinesterase were associated with a shorter cancer-specific survival. As we did not correlate CHE levels with the presence of liver metastasis it is not possible to clearly establish the cause for this correlation. However, low cholinesterase levels could indicate a systemic reaction occurring as a combined consequence of pancreatic cancer and liver insufficiency.

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