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Analysis of pancreatic islet and beta cell structure and function in patients with pancreatic lesions

Dissertation

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Für meine Großeltern, Eltern, Schwester, Familie

Neben dem Typ-1- und Typ-2-Diabetes mellitus rücken spezifischere Diabetesformen wie der Diabetes des exokrinen Pankreas (DEP) zunehmend in den Fokus der Forschung. Der DEP ist mit Läsionen des exokrinen Pankreas wie Neoplasien, Entzündungen und chirurgischen Resektionen assoziiert, die die normale Architektur oder Physiologie des Pankreas stören. Dadurch kommt es zu Beeinträchtigungen sowohl exokriner als auch endokriner Pankreasfunktionen. Der Zusammenhang zwischen Läsionen des exokrinen Pankreas und der Entwicklung einer diabetischen Stoffwechsellage bei Patienten mit DEP ist derzeit weitgehend ungeklärt. Ziel dieser Studie war es, den Glukosestoffwechsel bei Patienten mit Pankreasläsionen zu untersuchen, bei denen eine partielle oder totale Pankreatektomie geplant war. Insgesamt wurden 46 Pankreasproben von Patienten mit nicht-malignen Pankreasläsionen (NM) und duktalem Adenokarzinom des Pankreas (PDAC) analysiert, und in drei Kategorien eingeteilt: Patienten ohne Diabetes mellitus (ND), Patienten mit Typ-2-Diabetes (T2D) und Patienten mit DEP. Die morphologische Analyse der Langerhans-Inseln sowie der Insulin- und Glucagon-positiven Flächen wurde mit klinischen Parametern wie Alter, Body-Mass-Index (BMI), Diabetesdauer, HbA1c, Nüchtern-Glukose, Insulin, C-Peptid, Bilirubin, Lipase, Amylase, Pankreasamylase und C-reaktivem Protein (CRP) sowie mit dem *Homeostatic Model Assessment of Insulin Resistance und Beta-Cell Function* (HOMA-IR und -B) assoziiert. Besonderes Augenmerk wurde auf die Verteilung von Tumorstadium und -grad in den Patientengruppen und ihre Beziehung zu den morphologischen Merkmalen der Pankreasproben gelegt. Gruppenvergleiche wurden mittels *One-way Analysis of Variance* (ANOVA) durchgeführt und Unterschiede in der prozentualen Verteilung wurden mit dem *Fischer's exact test* bewertet. Assoziationen zwischen den Parametern wurden anhand von Korrelationskoeffizienten und entsprechenden p-Werten analysiert. Patienten mit DEP und T2D hatten höhere Nüchtern-Glukose- ($p < 0.001$), HbA1c- ($p < 0.001$) und HOMA-IR-Werte ($p < 0.05$), als Patienten ohne Diabetes mellitus. Auch bei der Verteilung von Tumorstadium und -grad wurden Unterschiede zwischen den Diabetesgruppen festgestellt. Die DEP-Gruppe hatte den höchsten Anteil an Tumoren im Stadium III/IV (ND: 10%, T2D: 0%, DEP: 30%; jeweils $p < 0.001$). Außerdem war der Anteil der G3-Tumore in der DEP-Gruppe höher als in der T2D- und ND-Gruppe (ND: 24 %, T2D: 43 %, DEP: 64 %; jeweils $p < 0.001$). Beim Vergleich der Tumor-Grading-Gruppen wies die G3-Gruppe signifikant höhere Nüchtern-Glukose- ($p < 0.05$) und HbA1c-Werte ($p < 0.05$) auf als die NM-Gruppe. Patienten mit DEP zeigten im Vergleich zur ND- und T2D-Gruppe eine reduzierte Glucagon-positive Fläche (jeweils $p < 0.05$) und im Vergleich zur ND-Gruppe eine kleinere Insulin-positive Fläche ($p < 0.05$). Sie wiesen zudem negative Korrelationen von Inselgröße und Nüchtern-Glukosespiegeln ($p < 0.05$), sowie absoluter Insulin-positiver Fläche und Nüchtern-Glukosespiegeln ($p < 0.001$) auf. Zusammenfassend weisen die Befunde darauf hin, dass bei Patienten mit PDAC der Schweregrad des Tumorgeschehens wesentlich das Ausmaß der Glukosestoffwechselstörung bestimmt und somit auch zur ausgeprägten Dysglykämie bei DEP beiträgt.

In addition to type 1 and type 2 diabetes, more specific forms of diabetes, such as diabetes of the exocrine pancreas (DEP), are receiving increasing attention in research. DEP is associated with damage to the exocrine pancreas caused by factors such as neoplasia, inflammation and surgical resection, all of which disrupt the normal structure and function of the pancreas. This results in a loss of both exocrine and endocrine function. The relationship between exocrine pancreatic lesions and the development of a diabetic metabolic state in patients with DEP remains poorly understood. The aim of this study was to investigate glucose metabolism in individuals with pancreatic lesions scheduled for partial or total pancreatectomy. A total of 46 pancreatic samples from patients with non-malignant pancreatic lesions (NM) and pancreatic ductal adenocarcinoma (PDAC) were analysed and divided into three categories: Patients without diabetes mellitus (ND), patients with type 2 diabetes (T2D) and patients with DEP. Morphological analysis of islets of Langerhans and insulin-positive areas was compared with clinical parameters such as age, body mass index (BMI), duration of diabetes, HbA1c, fasting glucose, insulin, C-peptide, bilirubin, lipase, amylase, pancreatic amylase and C-reactive protein (CRP), as well as the homeostatic model assessment of insulin resistance and beta-cell function (HOMA-IR and -B). Special attention was paid to the distribution of tumour stage and grade in the patients' groups and their relation to the morphological characteristics of the pancreatic samples. Group comparisons were made using one-way analysis of variance (ANOVA) and differences in percentage distributions were assessed using Fisher's exact test. Relationships between parameters were analysed using correlation coefficients and corresponding p-values. Patients with DEP and T2D had higher fasting glucose ($p < 0.001$), HbA1c ($p < 0.001$) and HOMA-IR levels ($p < 0.05$) than patients without diabetes mellitus. Differences were also observed in the distribution of tumour grading and stage between the diabetes groups. The DEP group had the highest proportion of stage III/IV tumours (ND: 10%, T2D: 0%, DEP: 30%; $p < 0.001$). In addition, the proportion of G3 tumours was higher in the DEP group than in the T2D and ND groups (ND: 24%, T2D: 43%, DEP: 64%; both $p < 0.001$). When comparing the tumour grading groups, the G3 group had significantly higher fasting glucose levels ($p < 0.05$) and HbA1c ($p < 0.05$) than the NM group. Patients with DEP showed a reduced glucagon-positive area compared to the ND and T2D groups (both $p < 0.05$) and a smaller insulin-positive area compared to the ND group ($p < 0.05$). They also showed negative correlations of islet size and fasting glucose levels ($p < 0.05$), as well as absolute insulin-positive area and fasting glucose levels ($p < 0.001$). In summary, the findings indicate that in patients with PDAC, the severity of tumour progression significantly determines the extent of glucose metabolism disturbances and thus also contributes to the pronounced dysglycaemia in DEP.

List of abbreviations

%	Percentage
<	Smaller than
+	Positive
°C	Degree Celsius
µl	Microlitre
ABC	Anatomical, Biological, Conditional
ADA	American Diabetes Association
AGEs	Advanced glycation end-products
AID	Automated Insulin Delivery
AJCC	American Joint Committee on Cancer
ANOVA	One-way Analysis of Variance
APC	Adenomatous polyposis coli gene
ATM	Ataxia-telangiectasia mutated
BD	Branch-duct
BMI	Body mass index
BRCA1	Breast Cancer 1 gene
BRCA2	Breast Cancer 2 gene
BSA	Bovine serum albumin
CA 19-9	Carbohydrate antigen 19-9
CDKN2A	Cyclin dependent kinase inhibitor 2A gene
CGM	Continuous glucose monitoring
cm^3	Cubic centimetre
CRP	C-reactive protein
CSII	Continuous Subcutaneous Insulin Infusion
CT	Computed tomography

DAB	Diaminobenzidine
DDG	Deutsche Diabetes Gesellschaft (German Diabetes Association)
DDZ	Deutsches Diabetes-Zentrum (German Diabetes Center)
DEP	Diabetes of the exocrine pancreas
DKA	Diabetic ketoacidosis
EASD	European Association for the Study of Diabetes
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediamine tetraacetate
e.g.	Exempli gratia
EPCAM	Epithelial cell adhesion molecule gene
FAMMM	Familial atypical multiple birthmark and melanoma syndrome
FAP	Familial adenomatous polyposis
FNK	Florence-Nightingale-Krankenhaus (Florence Nightingale Hospital)
FOLFIRINOX	Folinic acid, 5-fluorouracil, irinotecan, oxaliplatin
FPC	Familial pancreatic cancer
FPG	Fasting Plasma Glucose
G	Gram
GAD65	Glutamic acid decarboxylase 65
GLP-1	Glucagon-like Peptide-1
Gluc	Glucose
HbA1c	Haemoglobin A1c
HDL	High-density lipoprotein
HHS	Hyperosmolar hyperglycaemic syndrome

HLA	Human Leukocyte Antigen
HNPCC	Hereditary non-polyposis colorectal cancer syndrome
HOMA	Homeostatic Model Assessment
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMA-B	Homeostatic Model Assessment of Beta-Cell Function
HRP	Horseradish peroxidase
i.e.	Id est
i.s.	In serum
IA-2	Tyrosine phosphatase
IAA	Insulin autoantibodies
IAP	International Association of Pancreatology
ICA	Islet cell antibodies
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGF-1	Insulin-like growth factor-1
IGT	Impaired Glucose Tolerance
IPMN	Intraductal papillary mucinous neoplasia
IR	Insulin resistance
KDIGO	Kidney Disease Improving Global Outcomes
kg/m^2	Kilogram per square metre
LDL	Low-density lipoprotein
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
MASH	Metabolic Dysfunction-Associated Steatohepatitis
MCN	Li-Fraumeni syndrome
mg/dl	Milligrams per decilitre
MCN	Mucinous cystic neoplasia
MD	Main duct

Ml	Millilitre
MLH1	MutL homolog 1 gene
mmol/l	Millimoles per litre
mmol/mol	Millimoles per mole
MODY	Maturity-Onset Diabetes of the Young
MRI	Magnetic resonance imaging
MSH6	MutS homolog 6 gene
mU/l	Milliunits per litre
n	Number
Nab	Nanoparticle albumin-bound
NALIRIFOX	Liposomal irinotecan, 5-fluorouracil, leucovorin. oxaliplatin
ND	Patients with no diabetes mellitus
NDDG	National Diabetes Data Group
NM	Non-malignant
nmol/l	Nanomoles per litre
NCCN	National Comprehensive Cancer Network
oGTT	Oral glucose tolerance testing
p	Statistical significance
PanIN	Pancreatic intraepithelial neoplasia
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline
PDAC	Pancreatic ductal adenocarcinoma
PJS	Peutz-Jeghers syndrome
PMS2	Postmeiotic segregation increased 2 gene
r	Correlation coefficient
R0	No residual tumour

SEM	Standard Error of the Mean
SGLT2	Sodium-Glucose Transport Protein 2
STK11	Serine-threonine kinase 11 gene
TNM	Tumour, Nodes, Metastases
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TP53	Tumour protein 53 gene
U/l	Units per litre
UICC	Union for International Cancer Control
UK	United Kingdom
UKD	Universitätsklinikum Düsseldorf (University Hospital Düsseldorf)
USA	United States of America
VLDL	Very-low-density lipoprotein
vs.	versus
WHO	World Health Organisation
ZnT8	Zinc transporter 8

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1 Introduction

1.1 Diabetes mellitus

1.1.1 Definition and aetiology

"Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is both underutilized as an energy source and overproduced due to inappropriate gluconeogenesis and glycogenolysis, resulting in hyperglycaemia. Diabetes can be diagnosed by demonstrating increased concentrations of glucose in venous plasma or increased HbA1c in the blood" (American Diabetes Association 2024). Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurred vision, and weight loss. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs, especially of the eyes, kidneys, nerves, heart and blood vessels. The long-term effects of diabetes mellitus include progressive development of specific complications of retinopathy with potential blindness, neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic neuropathy as gastrointestinal and genitourinary dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Acute-term effects are life threatening consequences of uncontrolled hyperglycaemia as hyperglycaemia with ketoacidosis or non-ketotic hyperosmolar syndrome (American Diabetes Association 2024).

According to the American Diabetes Association classification, diabetes mellitus can be divided into four different categories based on their aetiology. An overview of the different diabetes types is given in Table 1A and Table 1B.

Table 1A: Classification of diabetes mellitus type 1 and type 2

TYPE OF DIABETES	ETIOLOGY	CHARACTERISTICS
Type 1 diabetes	Autoimmune (Type 1A) Idiopathic (Type 1B)	Autoimmune beta cell destruction that mostly leads to an absolute insulin deficiency
Type 2 diabetes	Genetic predisposition Life-style associated factors Comorbidity with other diseases Multifactorial	Can range from a predominant insulin resistance with a relative insulin deficiency to a largely secretory defect with insulin resistance

(American Diabetes Association 2024)

Table 2B: Classification of diabetes mellitus type 3 and type 4

TYPE OF DIABETES	ETIOLOGY	CHARACTERISTICS
Type 3 diabetes	Exocrine pancreatic disease	E.g. in the context of pancreatic neoplasia, pancreatitis, pancreatectomy, haemochromatosis, cystic fibrosis
	Genetic defects of beta cell function (e.g. MODY, neonatal diabetes, mitochondrial diabetes)	MODY: autosomal-dominant; diabetes in ≥ 3 generations; mutation of genes of transcription factors of beta cells or glucokinase; manifestation mostly during youth to early adulthood
	Genetic defects in insulin action	E.g. Type A insulin resistance, Leprechaunism, Rabson-Mendenhall syndrome, Lipoatrophic diabetes
	Endocrinopathies	E.g. Cushing's syndrome, acromegaly, phaeochromocytoma, hyperthyroidism, glucagonoma, somastostatinoma
	Medically-chemically induced	E.g. glucocorticoids, neuroleptics, interferon-alpha, pentamidine, thiazides, α-adrenergic agonists, beta-adrenergic agonists, thyroid hormone, ciclosporin, tacrolimus, L-asparaginase
	Infections	E.g. congenital rubella, cytomegalovirus
	Other genetic syndromes associated with diabetes	E.g. Down's syndrome, Turner's Syndrome, Klinefelter's syndrome, Prader-Willi syndrome, Wolfram's syndrome, Lawrence-Moon-Biedel syndrome, porphyria, Friedreich's ataxia, Huntington's chorea, myotonic dystrophy, Stiff-Man-Syndrome
	Uncommon forms of immune-mediated diabetes	
Type 4 diabetes	Gestational diabetes	Glucose tolerance disorder that occurs for the first time during pregnancy

MODY: Maturity-Onset Diabetes of the Young

(American Diabetes Association 2024)

1.1.2 Epidemiology

According to the International Diabetes Federation (IDF) worldwide about 537 million adults (about 10.5% of the global adult population), are currently living with diabetes mellitus and the number of people affected by the disease is still increasing (Sun et al. 2022). For 2045 the number is estimated to rise above 783 million (García-Chapa et al. 2017; Sun et al. 2022). Currently, about 1 in 10 adults has diabetes (Sun et al. 2022; Zheng et al. 2018). Diabetes mellitus is one of the fastest-growing health challenges of the 21st century, and its increasing prevalence is driven by a complex interplay of socioeconomic, demographic, nutritional, environmental and genetic factors. After adjusting for the impact of an ageing population, the prevalence of diabetes in adults nearly doubled worldwide between 1980 and 2014 (Kolb 2022). The increase was more pro-

nounced in low- and middle-income countries and in men compared with women (Kolb and Martin 2017). In 2021, the age-adjusted prevalence of diabetes mellitus in Europe was 61 million (Sun et al. 2022). In the global ranking, Germany is at the eighth position worldwide, counting 9.5 million people affected by diabetes. Diabetes mellitus is the ninth major cause of death and cardiovascular complications seem to be the leading cause for morbidity and mortality (Zheng et al. 2018). In 2021, approximately 6.7 million people died from diabetes and its comorbidities worldwide (Sun et al. 2022). Over the past decades, the prevalence of type 2 diabetes strongly increased in most regions of the world, reaching a proportion of 90-95% of all diabetes types (Henning 2018). This can mostly be explained by increased aging, urbanization and prevalence of a sedentary lifestyle, obesity and physical inactivity (Kolb and Martin 2017). In 2019, the IDF estimated 1.1 million children and adolescents (under 20 years) worldwide having type 1 diabetes (Patterson et al. 2019). There is some evidence that also type 2 diabetes is rising among children, mostly associated with increasing adiposity, but due to the lack of data, estimation of the number is still not possible (Sun et al. 2022). Although type 2 diabetes (90-95%) and type 1 diabetes (5-10%) are the most common forms, other diabetes types, as the diabetes of the exocrine pancreas (DEP) move into the focus of research. In a retrospective study, 1,868 patients diagnosed with diabetes mellitus and admitted to the University Hospital of Munich over the past 24 months were reclassified according to the American Diabetes Association's (ADA) diabetes classification. Upon reclassification, 9.2% of these patients were found to have DEP, although they had previously been misdiagnosed with type 2 diabetes (Ewald et al. 2012).

1.1.3 Type 1 diabetes

Approximately 5-10% of patients with diabetes mellitus are affected by type 1 diabetes, but the incidence and prevalence of this diabetes form is still increasing (Mobasseri et al. 2020). The pathogenesis of both, type 1 and type 2 diabetes is influenced by a variety of genetic and environmental factors that contribute to the progressive loss of beta cell mass and/or function, leading to clinically manifest hyperglycaemia (Patterson et al. 2019). In type 1 diabetes, the primary mechanism of progressive beta cell destruction is autoimmune-mediated, involving the immune cell-mediated destruction of pancreatic beta cells, which is associated with the presence of specific autoantibodies. Autoimmune markers associated with the development of type 1 diabetes include islet cell autoantibodies (ICA), glutamate decarboxylase 65 (GAD65) autoantibodies, insulin autoantibodies (IAA), tyrosine phosphatase IA-2 and IA-2beta autoantibodies (IA-2), and zinc transporter 8 (ZnT8) autoantibodies (Kawasaki 2023). The presence of one or more of these autoantibodies is observed in 85-90% of persons when fasting hyperglycaemia is first detected (American Diabetes Association 2024). The risk of developing type 1 diabetes increases with the number of relevant autoantibodies identified (Jacobsen et al. 2020). Type 1 diabetes is also strongly associated with HLA markers, in particular HLA-DQA, HLA-DQB, HLA-DQ8, HLA-

DR-3 and HLA-DR4. The highest risk appears to be conferred by the HLA DR-3/4 DQ8 genotype (Zielmann et al. 2022). In contrast, HLA-DQ6 appears to mediate protection against the development of type 1 diabetes (Sanjeevi 2000). However, beta cell destruction is associated not only with variable genetic predisposition, but also with environmental factors that are still poorly defined (Norris et al. 2020). Manifestation of type 1 diabetes is more frequently observed in childhood and adolescence but can also occur in adulthood. The rate of beta cell destruction is quite variable and appears to be related to age at diagnosis: mainly rapid in infants and children and mostly slow in adults (American Diabetes Association 2024). Type 1 diabetes is characterised by a high risk of diabetic ketoacidosis (DKA), which can occur at the first manifestation of the disease, especially in childhood (Calimag et al. 2023). Other common symptoms include polyuria, polydipsia, weight loss, dehydration, loss of appetite, slow wound healing, weakness, and fatigue. Stressful events, such as infections or surgery, may trigger disease manifestation. Additionally, there is an observed association between type 1 diabetes and other autoimmune disorders, including Hashimoto's thyroiditis, Graves' disease, Addison's disease, celiac disease, vitiligo, autoimmune hepatitis, myasthenia gravis, pernicious anaemia, and autoimmune polyglandular syndrome (American Diabetes Association 2024).

1.1.4 Type 2 diabetes

Type 2 diabetes is the most common form of diabetes mellitus. Type 2 diabetes affects 90-95% of people with diabetes, and its incidence and prevalence are still increasing. This increase appears to be related to environmental and lifestyle changes towards a western lifestyle characterized by a high energy diet and reduced physical activity, as well as the ageing of the population (Kolb and Martin 2017). Type 2 diabetes is a chronic, very heterogeneous, multifactorial, progressive disease characterized by inherited and/or acquired insulin resistance and qualitative and quantitative disturbances in insulin secretion (Petersmann et al. 2019). People with type 2 diabetes have relative insulin deficiency and peripheral insulin resistance. Although insulin levels may seem normal or even elevated in some instances, the hyperglycaemia observed would generally lead to even higher insulin levels. Studies also suggest that, in addition to insulin deficiency and insulin resistance, a decrease in beta cell mass may contribute to the pathogenesis of the disease (Eizirik et al. 2020). The role of alpha cells is also still object of research. Hyperglucagonemia has been described, but it is still unclear whether this is related to an increase in alpha cell mass (Campbell and Newgard 2021; Henquin and Rahier 2011). Nonetheless, alpha cell dysfunction seems to be a key factor in fasting hyperglycaemia and glucose dysregulation in type 2 diabetes. A better understanding of these pathways may be fundamental to the development of new therapeutic approaches. Insulin resistance in patients with type 2 diabetes leads to a marked reduction in glucose uptake in peripheral muscle tissue in response to insulin secretion. Increased hepatic glucose production despite fasting hyperinsulinemia has also been reported, indicating insulin resistance also

in the liver (Brunton 2016; DeFronzo et al. 2015). In addition, the inhibitory effect of insulin on lipolysis is reduced in patients with type 2 diabetes. The excess of free fatty acids causes lipotoxicity, which increases insulin resistance (Marušić et al. 2021). The incretin glucagon-like peptide 1 (GLP-1) is normally responsible for activating G-protein-coupled receptors on beta cells, stimulating insulin secretion in response to high glucose levels and regulating the postprandial insulin response. It also has an inhibitory effect on alpha cells, reducing glucagon secretion. In patients with type 2 diabetes, the effect of GLP-1 appears to be reduced, thereby contributing to the dysregulation of glucose homeostasis (Müller et al. 2019). Amylin, another hormone produced by beta cells, is reduced in type 2 diabetes due to beta cell insufficiency. Its role is to suppress glucagon release from pancreatic alpha cells, delay gastric emptying and increase satiety (Brunton 2016; DeFronzo et al. 2015). Patients with type 2 diabetes are more prone to develop a hyperosmolar hyperglycaemic syndrome (HHS) than DKA, which is more typical for type 1 diabetes, because residual insulin secretion, although reduced, still allows inhibition of peripheral lipolysis and thus ketone body production (Everett et al. 2023).

Several risk factors contribute to the complex pathogenesis of the disease. Genetic and environmental factors play an important role in the development of type 2 diabetes. Genetic predisposition and family history in first-degree relatives are common in type 2 diabetes. Studies have shown that about 39% of people with diabetes have a positive family history of the disease in at least one first-degree relative (Annis et al. 2005). The risk of developing type 2 diabetes is five to ten times higher for people with a family history of diabetes than for those without a positive family history (Sargeant et al. 2000; van 't Riet et al. 2010). Factors such as overweight, obesity, over-nutrition and physical inactivity are also important risk factors for the progression of the disease. Excess of weight indeed causes some degree of insulin resistance, inducing a higher necessity of insulin to overcome hyperglycaemia (Kolb 2022). Dietary intervention, weight reduction and increased physical activity may significantly improve insulin resistance. In a systematic review of 5 randomised controlled trials in people with type 2 diabetes, improvements in glycaemic control and insulin sensitivity were greater in persons on a Mediterranean diet than in those on a low-fat diet (Esposito et al. 2015; Ley et al. 2014). Epidemiological studies suggest that higher levels of physical activity are associated with a 30% reduction in diabetes risk (Ley et al. 2014). Furthermore, lifestyle interventions have proven effective. In a study involving individuals at high risk, a lifestyle intervention led to a 58% reduction in diabetes development over three to four years (Kolb and Martin 2017). This result underscores the importance of lifestyle modifications as both a preventive and therapeutic measure for type 2 diabetes. The risk of developing diabetes also increases with age, although the incidence and prevalence of type 2 diabetes in children and adolescents has increased dramatically in the last decade (American Diabetes Association 2024).

Additionally, an inverse association between type 2 diabetes and socioeconomic status has also been reported, as well as a positive association with increased exposure to residential traffic, noise and fine particulate matter (Kolb and Martin 2017). A list of other risk factors associated with type 2 diabetes is shown in Table 3.

Despite its high prevalence, type 2 diabetes often remains undiagnosed for years due to the absence of typical symptoms in its early stages. Nonetheless, even undiagnosed persons are at increased risk of developing diabetes-related complications (American Diabetes Association 2024).

Table 3: Risk factors for the development of type 2 diabetes

UNMODIFYABLE RISK FACTORS	MODIFYABLE RISK FACTORS
• Higher age • Male • Positive family history • Gestational diabetes in the history • Intrauterine development (foetal programming)	• Visceral obesity, BMI ≥ 25 kg/m² • Physical inactivity • High energy, low-fibre diet • High carbohydrate consumption • Diabetogenic drugs • Hypertension • Hyperuricemia • Dyslipidaemia • Fatty liver • Acanthosis nigricans • Obstructive sleep apnoea • Polycystic ovary syndrome • Gestational diabetes • Intrauterine growth retardation (IUGR) • Depression • Diabetogenic environment (e.g. fine dust, noise, socioeconomic status) • Smoking • Excessive alcohol consumption

BMI: Body mass index

(Fletcher et al. 2002; Girardin and Schwitzgebel 2007; Petersmann et al. 2019)

1.1.5 Diabetes of the exocrine pancreas

"Diabetes of the exocrine pancreas (DEP), previously defined as type 3c diabetes or secondary pancreatic diabetes, is thought to constitute 9% of diabetes in hospitalised patients. This form of diabetes results when a process, such as inflammation, neoplasia, or surgical resection, disrupts the global architecture or physiology of the pancreas, often resulting in both exocrine and endocrine dysfunction" (American Diabetes Association 2024; Hart et al. 2016; Woodmansey et al. 2017). DEP is usually characterised by a reduction in insulin production due to beta cell dysfunction and absolute beta cell loss, following exocrine pancreatic disease (American Diabetes Association 2024). However, not only beta cell loss has been reported, but alpha cell loss and loss of pancreatic polypeptide and somatostatin producing cells also appear to play a role (Andersen et al. 2017). This explains why patients with DEP often experience episodes of hyperglycaemia

alternating with episodes of hypoglycaemia. In fact, both insulin and glucagon production may be reduced. Failure to recognise this altered physiology may lead to suboptimal treatment. The risk of severe hypoglycaemia must be considered when establishing an insulin-based treatment regimen. Insulin resistance especially at early stages of the disease has also been shown to be typical of this type of diabetes (Sah et al. 2013). The combination of low levels of insulin, glucagon and pancreatic polypeptide contributes to rapid fluctuations in glucose levels. Previous studies have shown that DEP is often characterized by poorer glycaemic control and a significantly increased insulin requirement compared with patients with type 2 diabetes (Woodmansey et al. 2017). Insulin therapy is also often required at an earlier stage than in patients with type 2 diabetes, as beta cell dysfunction proceeds more rapidly in DEP (Hart et al. 2016). Additionally, because of malabsorption due to exocrine damage, pancreatic enzymes and vitamin D replacement should also be considered to prevent malnutrition and osteoporotic bone disease (Woodmansey et al. 2017; Wynne et al. 2019)

Diagnosis of the disease remains still a problem. It appears to be more common than has been previously considered. Data collected between 2005 and 2016 from the Royal College of General Practitioners Research and Surveillance Centre in the UK was used to assess the incidence of previous pancreatic disease in 31,789 adults newly diagnosed with diabetes. This retrospective analysis identified 559 cases of diabetes following pancreatic disease, with a median follow-up of 4.5 years from the date of pancreatic diagnosis. Of these patients, 87.8% were initially classified by clinicians as having type 2 diabetes, 7.7% as having T1D and only 2.7% were correctly identified as having DEP. Interestingly, when reclassified, the proportion of adults with DEP (1.8%) was higher than those with T1D (1.1%) (Woodmansey et al. 2017). As the clinical course of DEP differs from that of type 1 and type 2 diabetes, correct diagnosis is essential for optimal treatment and follow-up (Wynne et al. 2019).

The most common causes of DEP include both acute and chronic pancreatitis. However, other potential aetiologies include pancreatic neoplasia, post-pancreatectomy diabetes following surgical pancreatectomy, haemochromatosis, congenital agenesis of the pancreas, autoimmune pancreatitis, as well as traumatic and ischaemic pancreatitis. (Hart et al. 2016; Bhattamisra et al. 2019). The exact pathways involved in the development of the disease are still unclear and several mechanisms appear to be involved in the pathogenesis. Inflammatory mediators and fibrosis in the pancreatic tissue cause damage to both exocrine and endocrine functions, leading to a reduction in the size and function of the Langerhans islets and pancreatic enzymes (Wynne et al. 2019). The loss of pancreatic polypeptides, which have an influence on insulin receptor expression in the liver, can also lead to hepatic insulin resistance (Wynne et al. 2019). Para-neoplastic factors, such as increased cytokine release, seem to promote beta cell dysfunction and insulin resistance (Sah et al. 2013). Adrenomedullin, which is overexpressed in pancreatic ductal adenocarcinoma

(PDAC), may also be involved in the pathogenic process by acting as an inhibitor of insulin secretion and promoting invasive tumour behaviour (Aggarwal et al. 2013; Wynne et al. 2019). The contribution of elevated plasma islet amyloid polypeptide levels to insulin resistance is discussed controversially in the literature (Sah et al. 2013).

In general, patients with a history of pancreatic disease should be screened for both endocrine and exocrine pancreatic insufficiency. However, it remains unclear whether all patients with diabetes mellitus and concomitant exocrine pancreatic disease should be classified as having DEP, and strict diagnostic criteria have not yet been established. Hart et al. proposed the following criteria for the diagnosis of DEP:

- presence of diabetes mellitus
- presence of exocrine pancreatic disease, such as acute or chronic pancreatitis or pancreatic neoplasia
- a temporal relationship between the first diagnosis of exocrine pancreatic disease and the onset of diabetes mellitus (Hart et al. 2016).

Additionally, Ewald and Bretzel have proposed their own diagnostic criteria, as shown in Table 4 (Ewald and Bretzel 2013). The development of a consensus diagnostic tool to differentiate DEP from other diabetes subtypes may be necessary. In addition, further research is needed to evaluate current treatments and identify new therapeutic targets for more effective long-term management of DEP (Wynne et al. 2019).

Table 4: Diagnostic criteria for exocrine pancreatic disease by Ewald and Bretzel

CRITERIA	EXPRESSION
Major (all must be present)	• Diagnosis of diabetes mellitus • Exocrine pancreatic insufficiency (documented by stool tests for elastase-1 or a direct functional test) • Pathological imaging of the pancreas (endosonography, MRI, CT) • Lack of markers for type 1 diabetes
Minor criteria	• Impaired beta-cell function (e.g. HOMA-B, C-peptide glucose quotient) • No highly increased insulin resistance (e.g. HOMA-IR) • Reduced incretin secretion (e.g. GLP-1, pancreatic polypeptide) • Low serum values of fat-soluble vitamins (A, D, E and K)

(Ewald and Bretzel 2013)

1.1.6 Diagnosis of diabetes mellitus

Classification criteria for the diagnosis of diabetes mellitus have been established by the World Health Organization (WHO) and international diabetes committees such as the ADA, IDF, the European Association for the Study of Diabetes (EASD) and the US National Diabetes Data Group (NDDG) and have been adapted over the years to optimize the detection of the disease

(American Diabetes Association 2024; Patterson et al. 2019). Current recommendations use fasting plasma glucose (FPG) levels, the plasma glucose (PG) value after 2 hours during a 75 g oral glucose tolerance test (oGTT) and the HbA1c for diagnosis (American Diabetes Association 2024). The different criteria allow to differentiate between diabetes, prediabetes and normoglycaemia.

The definition of diabetes is given by following criteria:

- FPG of ≥ 126 mg/dl (or ≥ 7.0 mmol/l) after a fasting period of 8-12 hours
- PG value of ≥ 200 mg/dl (or ≥ 11.1 mmol/l) after 2 hours during an oGTT using a glucose load equivalent of 75 g of anhydrous glucose dissolved in water
- Random PG value of ≥ 200 mg/dl (or ≥ 11.1 mmol/l) in patients with classical hyperglycaemic symptoms (polyuria, polydipsia, weight loss) or in hyperglycaemic crisis
- HbA1c ≥ 6.5 % (or ≥ 48 mmol/mol Hb), with test performance in a laboratory using *National Glycohemoglobin Standardization Program* certified method and standardized to the Diabetes Control and Complications Trial Reference Method disease

In the absence of a typical clinical presentation, each diagnostic value must be confirmed by a second test, which may be performed on the same blood sample or on two different blood samples.

The definition of prediabetes is determined not only by the HbA1C value, but also by the presence of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Prediabetes can be diagnosed under the following circumstances:

- Presence of IFG: FPG in the range of 100-125 mg/dl (or 5.6-6.9 mmol/l)
- Presence of IGT: 2-hours PG level during oGTT in the range of 140-199 mg/dl (or 7.8-11.0 mmol/l) with fasting glucose values between 100-126 mg/dl (or 5.6-7.0 mmol/l)
- HbA1c value between 5.7-6.4 % (or 38-48 mmol/mol Hb)

Normoglycaemia is designated by:

- FPG <100 mg/dl (or < 5.6 mmol/l)
- 2h-PG oGTT <140 mg/dl (or 7.8 mmol/l)

If diabetes is diagnosed by measuring FPG, it is important to observe a fasting period of at least 8 hours to interpret the data correctly (Patterson et al. 2019).

1.1.7 Therapy of diabetes mellitus

As the present study focused mainly on type 2 diabetes and DEP, this section will focus on the treatment guidelines for these types of diabetes. In addition, as type 1 diabetes is another major form of diabetes, the treatment approaches for type 1 diabetes will also be described. Common milestones in the treatment of all three types of diabetes are pharmacological intervention with glucose-lowering drugs, nutritional education, physical exercise, self-monitoring of blood glucose levels and psychosocial support.

1.1.7.1 Therapy of type 1 diabetes

The indication for insulin therapy in type 1 diabetes is permanent and lifelong. The HbA1c target should be individualised for each patient based on life expectancy, comorbidities and risk of hypoglycaemia. The goal of therapy for type 1 diabetes is to prevent severe metabolic disturbances, such as severe hypoglycaemia or severe hyperglycaemia with ketoacidosis or diabetic coma, and to reduce the risk of developing microangiopathy and other long-term diabetes-related complications (Haak et al. 2024). According to insulin pharmacokinetics and pharmacodynamics, insulin therapy must cover both basal and prandial insulin needs. Basal insulin requirement is substituted with long-acting "basal insulin" or by Continuous Subcutaneous Insulin Infusion (CSII) via a pump. Prandial insulin needs are replaced by short-acting "bolus" insulin at mealtimes (basal-bolus principle) and to correct elevated glucose levels.

A conventional and an intensified strategy can be defined for insulin therapy. Conventional insulin therapy involves injecting fixed doses of premixed, i.e., a combination of short and long-acting insulin, in the morning and evening, as well as fixed times and carbohydrate portions for food intake. Simple conventional insulin therapy is only successful with a fixed meal plan. Insulin is usually given twice a day, at breakfast and dinner, and self-monitoring of blood glucose is recommended two times a day (Haak et al. 2024). The intensified strategy should be preferred to the conventional strategy (Haak et al. 2024; Pfohl et al. 2012).

The intensified strategy is characterised by the substitution of the basal insulin requirement and the substitution of the prandial insulin requirement at mealtimes. Insulin can be administered by insulin pen or pump. When insulin is administered by injection, the substitution of basal and prandial insulin is strictly separated. Long-acting insulin is given for basal needs and short-acting insulin for prandial needs. If the insulin is delivered by a pump, only a short-acting insulin is used for basal needs via CSII and for prandial needs via bolus application. Intensified insulin therapy allows the patient to individually determine the time and size of meals. Continuous Glucose Monitoring (CGM) systems should be offered to patients with type 1 diabetes for self-management, even if the precision is lower compared to laboratory measurements (Haak et al. 2024). If treatment goals are not reached with intensified insulin therapy and a CGM system, an insulin pump

therapy with Automated Insulin Delivery (AID) algorithm should be recommended. An AID system combines a CSII via an insulin pump, with a CGM system, through a computerized algorithm to enable automated adjustment of the insulin delivery rate (Morrison et al. 2022).

1.1.7.2 Therapy of type 2 diabetes

In people with type 2 diabetes, lifestyle interventions such as dietary education, weight loss, increased physical activity and smoking cessation are important treatment goals before the initiation of pharmacotherapy. A systematic review of eight meta-analyses and five randomised controlled trials by Esposito et al. examined the effect of a Mediterranean diet on the treatment of diabetes and prediabetes. It showed that in patients with diabetes, adherence to the Mediterranean diet was associated with lower HbA1c and a better cardiovascular risk factor profile compared with the control group (Esposito et al. 2015).

The Attica study revealed that following a Mediterranean diet led to improved fasting glucose homeostasis, insulin levels and reduced insulin resistance in both, normoglycaemic persons and patients with type 2 diabetes. Participants with high adherence to the Mediterranean diet showed a 15% reduction in basal glucose levels (Martín-Peláez et al. 2020; Panagiotakos et al. 2005). Another study by Toobert et al, involving 279 participants, showed a reduction in HbA1c by 0.4 percentage units in people who underwent a six-month intervention with a Mediterranean diet compared with the control group (Toobert et al. 2003).

The group of Su et al. randomised 30 patients with diabetic cardiovascular autonomic neuropathy into a control group treated with blood glucose lowering drugs only and an exercise group treated with blood glucose lowering drugs and resistance and aerobic training (Su et al. 2022). Compared with the control group, the exercise group showed a greater reduction in blood glucose levels and inflammatory markers and an improvement in autonomic nerve function (Su et al. 2022).

In addition to non-pharmacological therapy, pharmacotherapy must be evaluated after a detailed risk assessment to determine the choice and possible combination of antidiabetic and organ-protective drugs (Landgraf et al. 2024). Currently, the drug of choice is metformin, an oral biguanide, which must be dosed gradually, e.g. starting with 500 mg with the main meal and increasing by a further 500 mg each week to a total dose of 2 × 1000 mg per day (Landgraf et al. 2024). The combination of metformin with other drugs, such as sodium glucose transporter protein 2 (SGLT2) inhibitors or glucagon-like peptide-1 (GLP-1) receptor agonists, should be discussed in patients with cardiovascular or renal disease or with inadequate glycaemic control after 3 to 6 months of metformin monotherapy.

Combination therapy allows lower doses of each drug to be used and, in some cases, reduces side effects. If dual-combination therapy is not sufficient to achieve the target HbA1c values, triple therapy or combined insulin therapy should be added to the treatment regimen (Landgraf et al.

2024). Depending on the HbA1c at the time of diagnosis, it is also possible to start an extended treatment regimen directly. As in type 1 diabetes, the HbA1c target should be individualised for each patient based on life expectancy, comorbidities and risk of hypoglycaemia.

1.1.7.3 Therapy of diabetes of the exocrine pancreas

Metformin and insulin are the most used antidiabetic drugs in patients with DEP, although some guidelines recommend insulin alone for the treatment of DEP (Goodarzi and Petrov 2023; Shimizu et al. 2022). The choice of metformin or insulin as initial treatment is often based on individual patient characteristics. Metformin is regarded as a reasonable first choice for patients with mild hyperglycaemia and insulin resistance, whereas insulin is preferred for severe hyperglycaemia, especially in the setting of insulin deficiency (Goodarzi and Petrov 2023; Löhr et al. 2017). In recurrent cases, the therapy of patients initially treated with metformin needs to be intensified by administration of insulin as DEP progresses. In a case series conducted by Price et al., 38 patients with DEP and initial metformin monotherapy, required an insulin treatment within 12 months after diagnosis (Price et al. 2010). Apart from insulin and metformin, other antidiabetic medications have scarcely been studied for DEP management.

The HbA1c target for patients with DEP should not be less than 7% because patients with DEP experience not only beta cell loss, but also alpha cell as well as pancreatic polypeptide and somatostatin producing cell dysfunction with an increased risk of hypoglycaemia (Goodarzi and Petrov 2023). Failure to recognise this altered physiology may lead to suboptimal treatment.

Another aim of DEP therapy is to prevent malnutrition and osteoporosis due to exocrine insufficiency. Pancreatic enzymes and vitamin D replacement should also be considered in the management of DEP (Woodmansey et al. 2017; Wynne et al. 2019). As DEP is often misdiagnosed as type 2 diabetes, people with DEP frequently experience delayed insulin therapy and lack of pancreatic enzyme replacement. Accurate diagnosis of DEP is essential to ensure appropriate treatment and follow-up (Ewald et al. 2012; Wynne et al. 2019).

1.1.8 Complications of diabetes mellitus

People with diabetes are at increased risk of developing several serious health problems. In industrialised countries, diabetes appears to be the leading cause of cardiovascular disease, retinopathy, renal failure and lower limb amputation. Additionally, diabetes is associated with an increased risk of cancer and neurodegenerative diseases, such as vascular dementia, Alzheimer's disease and Parkinson's disease (Szablewski 2025). Regular monitoring of people with diabetes is therefore necessary (Magliano and Boyko 2021). The complications of diabetes mellitus can be divided into short-term and long-term complications (Petersmann et al. 2019).

1.1.8.1 Short-term complications of diabetes mellitus

Short-term complications of diabetes include an increased risk of infection due to the immunosuppressive effects of the disease (American Diabetes Association 2024). In addition, diabetes is associated with hyporeninaemic hypoaldosteronism, often associated with diabetic nephropathy, which can lead to hyperkalaemia (Sousa et al. 2016). Electrolyte disturbances, including depletion of potassium, sodium, magnesium and phosphate, are common in severe insulin deficiency or decompensated diabetes, such as DKA and non-ketotic HHS (Liamis et al. 2014).

DKA, a life-threatening complication more common in type 1 diabetes, is characterised by hyperglycaemia, metabolic acidosis and elevated ketones due to insulin deficiency (American Diabetes Association 2024). In contrast, HHS, which is more common in type 2 diabetes, involves severe hyperglycaemia, dehydration and hyperosmolality without significant ketoacidosis (Petersmann et al. 2019). Hypoglycaemia is another serious short-term complication, usually caused by insulin overdose, excessive glucose consumption, low carbohydrate intake or alcohol use. It can lead to neuroglycopenic symptoms such as impaired consciousness or seizures and is more common in type 1 diabetes, typically occurring when glucose levels fall below 2.8-3.0 mmol/L (Cryer 2010).

1.1.8.2 Long-term complications of diabetes mellitus

Long-term complications affect organs such as the heart, blood vessels, kidneys, nerves, eyes and skin. Diabetes significantly increases the risk of cardiovascular complications, including acute myocardial infarction, stroke, heart failure and peripheral arterial disease. Indeed, patients with diabetes are more likely to develop dyslipidaemia, with elevated VLDL and LDL cholesterol and reduced HDL cholesterol, which increases the risk not only of metabolic abnormalities such as metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH), but also of cardiovascular disease (Bahiru et al. 2021). Macroangiopathy, the leading cause of death in people with diabetes, reduces life expectancy by 12 years compared with people without diabetes (Hajar 2017). The risk of peripheral artery disease is two to four times higher in people with diabetes. The use of dapagliflozin, an SGLT2 inhibitor, has been associated with reduced rates of cardiovascular death and hospitalisation for heart failure, regardless of the presence or absence of diabetes (Kohlmorgen et al. 2021; McMurray et al. 2019; Wiviott et al. 2019).

Microangiopathy refers to diabetes-induced damage to small blood vessels. Although the exact mechanisms remain unclear, several factors are thought to contribute to the development of microvascular complications. These include oxidative stress, hyperosmolar stress and inflammation induced by advanced glycation end products (AGEs) and toll-like receptor activation. AGEs are a diverse group of molecules formed by the non-enzymatic reaction between reducing sugars and

the amino groups of proteins, lipids and nucleic acids. When AGEs interact with their cell-bound receptors, they generate oxygen radicals, pro-inflammatory cytokines and cell adhesion molecules, all of which are able to promote inflammation and tissue damage (Prasad and Tiwari 2017). In addition, toll-like receptors, which are critical for immune activation by recognising microbial products such as viral DNA, bacteria and fungi, can exacerbate the condition by triggering the excessive release of immune mediators. Together, these processes are thought to play an important role in the onset and progression of microangiopathy in diabetes (Beutler 2002; Madonna et al. 2017).

Another long-term complication of diabetes is diabetic nephropathy which represents a leading cause of end-stage renal disease in both developed and developing countries. The extent of kidney failure is determined by the KDIGO (Kidney Disease Improving Global Outcomes) criteria, including glomerular filtration rate, albuminuria and proteinuria. Kidney biopsies have shown that pathological changes mainly affect the glomerulus, with diffuse and nodular mesangial proliferation (Navaneethan et al. 2023; Qi et al. 2017).

Diabetic neuropathy can affect both, the sensorimotor and autonomic nervous systems. About 30% of people with diabetes develop diabetic sensorimotor polyneuropathy. This condition can lead to complications such as neuropathic foot syndrome, Charcot neuroosteoarthropathy and amputation. Diabetic autonomic neuropathy affects organs controlled by the autonomic nervous system, with common manifestations including cardiovascular problems (e.g. tachycardia and hypotension), gastrointestinal symptoms (e.g. gastroparesis), bladder dysfunction and unawareness of hypoglycaemia. Treatment goals focus on glycaemic control, pain management and slowing the progression of neuropathy (Cole and Florez 2020).

Diabetic foot syndrome includes foot ulcers, deformities (e.g. Charcot foot) and amputations, often as a result of trauma due to diabetic polyneuropathy. More than 50% of patients with diabetic foot syndrome also have peripheral arterial disease (Volmer-Thole and Lobmann 2016).

Diabetes mellitus is also a major risk factor for depression and neurodegenerative diseases, including vascular dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. Although the exact mechanisms are not fully understood, it is believed that hyperglycaemia and hyperinsulinaemia contribute to pathological changes, including mitochondrial dysfunction, oxidative stress and inflammatory responses (Szablewski 2025).

Several studies have reported a significantly higher risk of developing various types of cancer in people with diabetes mellitus. In a longitudinal retrospective cohort study by Chang et al., patients with diabetes had a 20% higher risk of developing cancer compared with patients without diabetes. The highest hazard ratio was observed for cancer of the liver and pancreas, followed by a moderately increased risk for oral, colorectal, gallbladder, kidney and brain cancer. Conversely,

patients with diabetes had a lower risk of developing oesophageal cancer than those without diabetes (Chang et al. 2024).

Diabetic retinopathy and maculopathy are major causes of vision loss in the developed world. Early stages include non-proliferative retinopathy with microaneurysms and haemorrhages, progressing to proliferative retinopathy with neovascularisation and retinal detachment (Lin et al. 2021).

Oral and dermal complications such as frequent bacterial and fungal infections and impaired wound healing are common in diabetes. Cutaneous manifestations, particularly in type 2 diabetes, include *pseudoacanthosis nigricans*, *necrobiosis lipoidica* and xanthelasma (American Diabetes Association 2024).

1.2 Morphology of the human exocrine and endocrine pancreas

Macroscopically, the pancreas can be divided into three parts: the head, the body and the tail. Some authors also distinguish two other structures, that make up the head of the pancreas: the uncinate process, located below the superior mesenteric artery and the isthmus, located above the superior mesenteric artery (Dolenšek et al. 2015). The average estimated size of the pancreas is 14-18 cm long, two to nine cm wide and two to three cm thick. Histologically, the pancreas can be divided in an exocrine and endocrine pancreas.

The exocrine pancreas accounts for 96-99% of the total pancreatic volume. It is made up of several glandular lobules, each measuring about one to ten mm in diameter. Each lobule is made up of structures called acini. The acini consist of pyramidal epithelial cells, the acinar cells, which have a broad basal cell pole and a narrow apical cell pole. They are arranged in a layer concentrically around the small central glandular lumen. Secretions drain from the lumen into an intercalated duct and from there into the intralobular and interlobular ducts, which converge in the main pancreatic duct (also called the duct of Wirsung). The main pancreatic duct drains into the duodenum with the common bile duct via the major duodenal papilla (also called Vater's papilla). Proximal to the main pancreatic duct, an accessory duct (also called the duct of Santorini), which is a remnant of the dorsal pancreas during organogenesis, enters the duodenum via the minor duodenal papilla. The purpose of the exocrine pancreas is to secrete digestive enzymes such as the endopeptidase’s trypsinogen, chymotrypsinogen, elastase, the exopeptidases carboxypeptidase and aminopeptidase, pancreatic lipase, phospholipase A and cholinesterase. In addition, the acinar cells produce bicarbonate, which plays an important role in neutralising gastric acid in the duodenum. Dysfunction of the exocrine pancreas may result in malabsorption and malnutrition (Dolenšek et al. 2015).

The endocrine pancreas makes up 1-4% of the total volume of the pancreas and its purpose is to produce hormones. It consists of endocrine microorgans called islets of Langerhans, which are composed of thousands of endocrine cells. In addition to the islets of Langerhans, single endocrine cells can be found in the acinar and ductal tissue. The islets include several types of endocrine cells: beta, alpha, delta, epsilon, and gamma cells. Beta cells, responsible for insulin secretion, are the most abundant, occupying 50–70% of the islet area. Alpha cells, which secrete glucagon, account for 20–40%. Delta and gamma cells, producing somatostatin and pancreatic polypeptide respectively, contribute less than 10%. Epsilon cells are the least common, comprising under 1% of the islet and releasing ghrelin. Beta cells are mainly found in the core of the islet, but they can also be arranged in clusters in a ribbon-like pattern or distributed throughout the islet. Studies have shown that smaller islets, with a diameter of less than 100 µm, tend to have a mantle-core organisation of cells, while larger islets have a more complex arrangement of endocrine cells (Dolenšek et al. 2015; Dybala and Hara 2019). The total number of islets of Langerhans in humans appears to be around 1,000,000-15,000,000 with an estimated total mass of 0.5-1.5 g (Dolenšek et al. 2015). Studies have shown that the density of islets per unit volume is similar in the head and body of the pancreas, and twice as high in the tail (Wang et al. 2013). The head of the pancreas has a higher number of smaller islets, while the tail has a smaller number of larger islets. The micro-organisation of the islets seems to be constant in the different parts of the pancreas, except for the posterior part of the head, which is rich in gamma cells and poor in alpha and beta cells. A study by Wang et al. showed that in patients with type 2 diabetes, the loss of insulin-producing beta cells in the head of the pancreas is more pronounced than in the body and tail (Wang et al. 2013).

The physiological functions of both the exocrine and endocrine pancreas can be significantly impaired by various factors, including pancreatitis, pancreatic surgery or resection, trauma, and pancreatic tumours.

1.3 Pancreas Neoplasia

1.3.1 Epidemiology

Over 95 % of pancreatic carcinomas are PDAC, caused by malignant degeneration of the exocrine part of the pancreas. Endocrine tumours, which originate from the endocrine cells of the islets of Langerhans (e.g. insulinoma, glucagonoma), are less common. In Germany, pancreatic exocrine cancer ranks 6th among newly diagnosed cancers and affects men and women more or less equally. The average age at diagnosis is usually 72 years for men and 76 years for women. In 2021, pancreatic exocrine cancer was the 4th most common cause of death among all cancers, accounting for 7.5% of cancer deaths in men and 9% of cancer deaths in women. Accordingly, the relative 5-year survival rate for pancreatic cancer of 11% for men and women is one of the

lowest survival rates of all cancers in Germany. Reasons for the poor prognosis include late diagnosis, the resulting low curative resection rate, and the early and aggressive metastatic behaviour (Seufferlein et al. 2024).

1.3.2 Risk factors

Lifestyle factors such as smoking, excessive alcohol consumption and adiposity are known risk factors for exocrine pancreatic cancer. According to a meta-analysis of 19 prospective studies involving a total of 4,211,129 people, drinking more than 15 g of alcohol per day is associated with an increased risk of pancreatic cancer. In people with high alcohol consumption, ≥ 24 g per day, the relative risk was 1.15. With high-proof alcohol consumption, the relative risk raised to 1.43 (Wang et al. 2016). For smokers, the calculated relative risk of developing pancreatic cancer was 1.8 compared with non-smokers. Years of smoking and number of cigarettes smoked per day correlated with an increased risk. On the other hand, the relative risk can be reduced rapidly by quitting smoking. The study by Lugo A. et al. showed a reduction in relative risk to 0.7 after ten years of abstinence compared with active smokers. After twenty years of abstinence, the relative risk was comparable to that of never smokers (Lugo et al. 2018). According to the analysis by Silveira E.A. et al, visceral adiposity increases the risk of pancreatic cancer to a relative risk of 1.19 (Silveira et al. 2021). In addition, studies have shown that each 5 kg/m^2 increase in BMI up to the age of thirty was associated with an augmented relative risk of 1.17 (Hidayat et al. 2018).

Diabetes mellitus also appears to be associated with an increased risk of pancreatic cancer. However, the relationship appears to be bidirectional. While diabetes may contribute to the development of pancreatic cancer, pancreatic cancer itself can also disturb glucose metabolism. A study by Zhang J. J. et al. showed a negative correlation between the duration of diabetes and risk of pancreatic cancer. An increased risk of developing pancreatic cancer was found in patients with new onset diabetes mellitus within the first two years after diabetes diagnosis. Ten years after the onset of diabetes, there was no longer a statistically significant increased risk for cancer (Zhang et al. 2019). On the other hand, elevated blood glucose levels may be one of the earliest signs of pancreatic cancer. Studies have shown that among patients with PDAC who also have diabetes, 74-88% were diagnosed with diabetes less than 24 months before their cancer diagnosis (Aggarwal et al, 2013; Andersen et al, 2017). This suggests that in many cases, new-onset diabetes may be induced by the tumour and could serve as a potential marker for early detection of pancreatic cancer. Further evidence comes from a study by Pannala et al. including 104 patients who underwent surgical resection for pancreatic cancer, 41 of whom had diabetes at the time of surgery. Of those with new-onset diabetes, 57% experienced a reversal of their diabetes following tumour removal. In contrast, in all patients with long-standing diabetes, the condition persisted after pancreatic resection (Pannala et al. 2008).

In addition to diabetes, non-hereditary chronic pancreatitis is also a risk factor for the development of pancreatic tumours (Beyer et al. 2022). A report by the International Pancreatitis Study Group showed a cumulative risk of 1.8 % over ten years and a risk of 4 % over twenty years (Lowenfels et al. 1993).

In addition to lifestyle factors and pre-existing conditions, genetic predisposition also appears to play a role in the development of pancreatic cancer. The term familial pancreatic cancer (FPC) is used when two first-degree blood relatives, at least one of whom is a first-degree relative of the person being tested, have developed pancreatic cancer, and when two or more blood relatives on the same side of the family have developed pancreatic cancer, one of whom is a first-degree relative of the person being tested. No pathogenic germline variant can be detected in patients with FPC. FPC should be distinguished from genetic tumour risk syndromes with an increased risk of pancreatic cancer. Patients suspected of having a genetic tumour syndrome should be offered germline analysis. Examples of genetic tumour syndromes associated with an increased risk of pancreatic cancer include familial adenomatous polyposis (FAP, APC gene), ataxia-telangiectasia mutated (ATM, ATM gene), familial breast and ovarian cancer syndrome (BRCA2 gene), hereditary breast and ovarian cancer syndrome (BRCA1 gene), familial atypical multiple birthmark and melanoma syndrome (FAMMM, CDKN2A gene), Lynch or hereditary non-polyposis colorectal cancer syndrome (HNPCC, MLH1, MSH2, MSH6, PMS2, EPCAM gene), Peutz-Jeghers syndrome (PJS, STK11 gene), Li-Fraumeni syndrome (LFS, TP53 gene) (Seufferlein et al. 2024).

1.3.3 Therapy of pancreas carcinoma

Surgery is the only potentially curative treatment for pancreatic cancer. Surgical resection should be the treatment of choice if metastatic disease has been excluded (Seufferlein et al. 2024). Diagnostic procedures include abdominal ultrasound, computed tomography, magnetic resonance imaging and endosonography. According to the International Association of Pancreatology (IAP) consensus and the ABC criteria for resectability, diagnostic laparoscopy is recommended before laparotomy if the tumour is > 3 cm, the tumour maker CA 19-9 > 500 U/ml or in the presence of ascites (excluding hepatic or portal vein cause), especially if no metastases are visible by imaging techniques (Ta et al. 2019). Regarding tumour size, studies have shown that a tumour size of > 3 cm combined with an unintentional weight loss of more than 5 kg increases the risk of organ metastases to 30% (Isaji et al. 2018). In up to 20% of cases, diagnostic laparoscopy can reveal previously occult metastases in the peritoneum and/or liver that are not visible by imaging due to their size (Seufferlein et al. 2024). The goal of surgical therapy is to achieve an R0 resection. An R0 situation means that no cancer cells can be definitively detected microscopically at the resection margin. The anatomical resectability of pancreatic carcinoma with respect to locoregional vascular involvement should be assessed by contrast-enhanced CT according to the criteria of the

National Comprehensive Cancer Network (NCCN). An overview of the NCCN criteria is provided in Table 5.

Table 5: Classification of anatomical resectability in pancreatic cancer according to the criteria of the National Comprehensive Cancer Network

RESECTABILITY	ARTERIAL SYSTEM	VENOUS SYSTEM
Resectable	No tumour contact with the coeliac trunk (CT), superior mesenteric artery (SMA) or common hepatic artery (CHA)	No tumour contact with the superior mesenteric vein (SMV) or portal vein (PV) or ≤ 180° contact without contour irregularity of the vein
Borderline-resectable	• Tumour in the head of the pancreas or uncinate process: • Solid tumour contact with the CHA without extension to the CT or the bifurcation of the hepatic artery • Solid tumour contact with the SMA by ≤ 180° • Solid tumour contact with norm variant artery • Tumour in the pancreatic body and tail: • Solid tumour contact with the CT by ≤ 180° • Solid tumour contact with the CT by > 180° without infiltration of the aorta and with intact gastroduodenal artery	• Solid tumour contact with the inferior vena cava • Solid tumour contact with the SMV or PV by > 180° • Solid tumour contact with the SMV or PV by ≤ 180° with vein contour irregularity or thrombosis of the vein with preserved vein proximal and distal to the affected vascular segment
Not resectable	<u>Tumour in the head of the pancreas or uncinate process:</u> • Solid tumour contact with the SMA by > 180° • Solid tumour contact with the CT by > 180° <u>Tumour in the pancreatic body and tail:</u> • Solid tumour contact with the SMA by > 180° • Solid tumour contact with the CT by > 180° • Solid tumour contact with the CT and infiltration of the aorta	<u>Tumour in the head of the pancreas or uncinate process:</u> • Non-reconstructable SMV or PV due to tumour infiltration or occlusion • Tumour contact with the most proximal jejunal branch draining into the portal vein <u>Tumour in the pancreatic body and tail:</u> • Non-reconstructable SMV or PV due to tumour infiltration or occlusion

CT: coeliac trunk, SMA: superior mesenteric artery, CHA: common hepatic artery, SMV: superior mesenteric vein, PV: portal vein

(Isaji et al. 2018; Persigehl et al. 2020; Seufferlein et al. 2024)

The surgical procedure differs depending on the location of the tumour. For pancreatic head cancer, the resection is usually a partial duodenopancreatectomy (also known as Whipple procedure) with or without preservation of the pylorus. In rare cases, total pancreatectomy may be necessary if the carcinoma extends to the left side. If necessary, the resection should be extended if there is infiltration of neighbouring organs and other structures. The surgical procedure for pancreatic tail cancer is left pancreatectomy. Cancer of the pancreatic corpus usually requires a resection of the left pancreas or, if necessary, a total duodenopancreatectomy. In all three cases, the surgical procedure involves a regional lymphadenectomy with resection of at least 12 regional lymph nodes

(Seufferlein et al. 2024). After resection, the tumour is classified according to the TNM (Tumour, Nodes, Metastases) classification, tumour grading and UICC (Union for International Cancer Control) classification for prognostic purposes. The TNM/UICC classification of pancreatic cancer is represented in Table 5.

Table 6: UICC and TNM classification of pancreatic cancer (8th edition)

UICC-Staging	T-Stage	N-Stage	M-Stage
0	**Tis**: carcinoma in situ	**N0:** no presence of regional lymph node metastases	**M0**: no presence of distant metastases
IA	**T1**: limited to the pancreas, tumour ≤ 2 cm in greatest dimension	**N0**	**M0**
IB	**T2**: limited to the pancreas, tumour ≥ 2 cm in greatest dimension	**N0**	**M0**
IIA	**T3**: tumour ≥ 4 cm in greatest dimension	**N0**	**M0**
IIB	**T1-T3**	**N1**: presence of regional lymph node metastases	**M0**
III	**T4**: tumour involves coeliac axis, superior mesenteric artery and/or common hepatic artery regardless of size	**N0-N1**	**M0**
IV	**T1-T4**	**N0-N1**	**M1**: presence of distant metastases

UICC: Union for International Cancer Control; TNM: Tumour, regional lymph nodes, metastasis

(Seufferlein et al. 2024)

Due to the increased risk of recurrence and reduced life expectancy after surgical resection alone, multimodal therapy is recommended for the treatment of pancreatic cancer. For example, adjuvant chemotherapy should be given after R0 resection of UICC stage I-III pancreatic cancer. Possible chemotherapy agents include FOLFIRINOX as a combination therapy (consisting of folinic acid, 5-fluorouracil, irinotecan, oxaliplatin) for patients in good general condition (ECOG 0-1). In the study of Conroy et al., combination chemotherapy with modified-FOLFIRINOX lead to a significant improvement in recurrence-free survival, overall survival and 3-year survival compared to gemcitabine monotherapy. The median overall survival was 54.4 months in the modified-FOLFIRINOX group and 35.0 months in the gemcitabine group and the disease-free survival rate at 3 years was 39.7% in the modified-FOLFIRINOX group and 21.4% in the gemcitabine group (Conroy et al. 2018). For patients with reduced general conditions (ECOG 1-2), gemcitabine monotherapy or gemcitabine/capecitabine combination therapy is recommended. The recommended duration of adjuvant chemotherapy is 6 months.

Patients with borderline resectable pancreatic cancer should be offered neoadjuvant therapy in the form of preoperative chemotherapy or chemoradiotherapy. In the study by Verstaijne et al., 246 patients with resectable and borderline resectable pancreatic cancer were randomised to neoadjuvant chemoradiotherapy (n = 119) or upfront surgery (n = 127). At a median follow-up of 59 months, the 5-year overall survival rate was 20.5% with neoadjuvant chemoradiotherapy and 6.5% with upfront surgery (Versteijne et al. 2022).

First-line chemotherapy is recommended for pancreatic cancer diagnosed as unresectable. The primary goal is to return the tumour to a resectable state after chemotherapy. First-line chemotherapy combinations are FOLFIRINOX and gemcitabine + nab-paclitaxel. Studies have shown tumour responses of around 30% in patients with metastatic pancreatic cancer treated with FOLFIRINOX and gemcitabine + nab-paclitaxel compared to 10% with gemcitabine monotherapy (Conroy et al. 2018; Hoff et al. 2013). Patients with ECOG 2 who are ineligible for first-line FOLFIRINOX may receive a reduced dose of gemcitabine + nab-paclitaxel (Macarulla et al. 2019; Wainberg et al. 2023).

For metastatic or locally advanced pancreatic cancer, palliative chemotherapy is recommended when ECOG performance status is 0 to 2 to improve quality of life, clinical benefit and survival. Palliative chemotherapy regimens include FOLFIRINOX, NALIRIFOX (off-label use), gemcitabine + nab-paclitaxel and gemcitabine + erlotinib, and gemcitabine monotherapy (Seufferlein et al. 2024). In the presence of a germline BRCA-1/2 mutation, platinum-based pre-treatment for at least 16 weeks and the PARP inhibitor Olaparib as maintenance therapy is a possible treatment option (Seufferlein et al. 2024). In addition to surgery and chemotherapy, psycho-oncological, psychosocial, nutritional and palliative care measures play an important role in the treatment of patients with pancreatic cancer.

1.3.4 Non-malignant pancreatic tumours

The most common benign pancreatic neoplasm is serous cystadenoma, a cystic lesion of the pancreas that is more common in older women over the age of 60. One third of patients are asymptomatic, while the remainder present with non-specific complaints such as abdominal pain, vomiting and fever (Hruban et al. 2007).

Intraductal papillary mucinous neoplasia (IPMN), mucinous cystic neoplasia (MCN) and pancreatic intraepithelial neoplasia (PanIN) are the so-called premalignant pancreatic neoplasms, i.e. lesions with the potential for malignant degeneration. Ductal adenocarcinoma develops mainly from these three precursor lesions (Hruban et al. 2007). IPMNs are the most common type of pancreatic cyst and can be divided into main duct (MD)-IPMN and branch duct (BD)-IPMN. BD-IPMNs are the most common IPMNs and involve the side branches of the pancreatic ductal system, but not the main pancreatic duct. BD-IPMNs are mostly found in the head and tail of the

pancreas (70%), with the remainder located in the body and tail, and have a lower risk of degeneration compared to MD-IPMNs (Gardner et al. 2024). MD-IPMNs are confined to the main pancreatic duct. Approximately 65% of MD-IPMNs become malignant over time and are the IPMN subtype most likely to cause attributable symptoms such as acute pancreatitis or abdominal pain (Stark et al. 2016). Mixed-type-IPMNs involve both the main and accessory pancreatic ducts and, like MD-IPMNs, have a higher risk of malignant transformation and are often symptomatic (Sohn et al. 2004).

MCNs are mucin-producing neoplasms with a malignant potential comparable to that of IPMNs. MCNs usually occur in middle-aged women and are located in the body and tail of the pancreas. They present as sharply demarcated cystic lesions and unlike IPMNs, they do not involve the main pancreatic duct (Liang et al. 2021).

PanINs are pre-invasive lesions that are considered precursors to ductal adenocarcinoma. They are mostly located in the head of the pancreas, arise in the smaller pancreatic ducts and are less than 0.5 cm in size (Hruban et al. 2007). PanINs were originally classified as PanIN-1A or PanIN-1B and PanIN-2 or PanIN-3 according to the degree of architectural and cytonuclear abnormalities but are now known to be divided into low-grade and high-grade PanINs. A study by Andea et al. showed a progressive increase in the number and grade of PanINs when comparing patients in the healthy control group with normal pancreatic function with patients with chronic pancreatitis and with ductal adenocarcinoma. PanINs were identified in 16% of control patients, 60% of chronic pancreatitis patients and 82% of pancreatic cancer patients (Andea et al. 2003).

1.4 Glucose metabolism in patients with pancreatic cancer

Alterations in glucose metabolism in patients with PDAC have been described in many studies. Type 2 diabetes appears to be a risk factor for the development of PDAC, but controversially, PDAC also appears to lead to alterations in glucose metabolism. In a retrospective study of 100 patients diagnosed with cancers of the lung, breast, colon, prostate or pancreas, 68% of patients with PDAC had concurrent diabetes, whereas the prevalence of diabetes in the other age-matched cancer cohorts ranged from 15 to 21% (Aggarwal et al. 2013). Importantly, among patients with PDAC who also have diabetes, 74-88% of patients were diagnosed with diabetes less than 24 months before their PDAC diagnosis (Aggarwal et al. 2013; Andersen et al. 2017). This suggests that in many patients, new-onset diabetes is caused by the tumour and may be useful in early diagnosis of PDAC (Aggarwal et al. 2013; Andersen et al. 2017). Diabetes and PDAC seems to have a "dual causality", in that both long-standing type 2 diabetes is a risk factor for the development of PDAC and, conversely, PDAC is a potential cause of diabetes in many cases (Andersen et al. 2017).

However, the exact mechanisms linking PDAC and diabetes are still poorly understood. Both insulin resistance and beta cell loss are thought to play a role in the development of diabetes secondary to PDAC (Sah et al. 2013). Unlike in other types of diabetes, islet loss in diabetes secondary to PDAC affects not only beta cells but also pancreatic polypeptide cells in the early stages of the disease and alpha cells in the late stages (Andersen et al. 2017). The concomitant exocrine pancreatic insufficiency also leads to maldigestion and impaired incretin secretion (Andersen et al. 2017; Dominguez-Muñoz et al. 2024). Typically, beta cell secretory capacity is preserved until most of the exocrine function of the pancreas is lost (Andersen et al. 2017).

In a study by Pannala et al., 512 patients with PDAC were compared with 933 controls. Diabetes was found in 47% of PDAC patients compared to only 7% of controls. Normal fasting glucose was found in only 14% of PDAC patients and 59% of controls (Pannala et al. 2008). These observations strongly suggest that new-onset diabetes associated with PDAC may be due to paraneoplastic mechanisms that disrupt insulin secretion or action, leading to diabetes. A study conducted by the Mayo Clinic (MN, Rochester, USA) and the MD Anderson Cancer Center (TX, Houston, USA) identified overexpression of adrenomedullin, a 52 amino acid peptide, as a possible mechanism for the development of DEP. Adrenomedullin was shown to mediate pancreatic cancer-induced inhibition of insulin secretion in beta cells. The plasma concentration of adrenomedullin was found to be higher in patients with PDAC compared to controls, and the concentration was even higher in patients with pancreatic cancer-induced diabetes (Aggarwal et al. 2013; Sah et al. 2013).

A study by Javeed et al. showed that in PDAC, exosomes contain high levels of adrenomedullin, which is able to enter beta cells via caveolin-mediated endocytosis or macropinocytosis and inhibits insulin secretion (Javeed et al. 2015). Another study describes the gap junction protein connexin26, a known tumour suppressor, as a possible player in the induction of glucose intolerance in PDAC. Connexin26 was found to be 10.8 and 6.9 times more abundant in pancreatic cancer with diabetes than in normal pancreas and chronic pancreatitis respectively and was predominantly localised to the islets in the vicinity of the pancreatic cancer tissue (Pfeffer et al. 2004).

Basso et al. identified a hyperglycaemic effect of the 14-amino-acid N-terminal peptide of S100A8 produced by pancreatic tumour cells and monocytes by activating inflammation pathways, while Huang et al. recognised an overexpression of the genes encoding vanin-1 and matrix metalloproteinase 9 in PDAC associated with diabetes and proposed them as useful biomarkers for disease detection (Basso et al. 2006; Huang et al. 2010). However, further studies are needed to understand the mechanisms leading from PDAC to DEP.

1.5 Aim of the study

The aim of this study is to contribute to the understanding of the relationship between impaired glucose metabolism and pancreatic neoplasia, with a focus on PDAC. While an association between diabetes mellitus and PDAC has been widely reported in the literature, the mechanisms leading to pancreatic endocrine dysfunction after exocrine disease remain unclear. This study therefore aims to identify associations between clinical parameters and morphological characteristics of the pancreatic islets and their insulin- and glucagon-positive areas in three different groups of PDAC-bearing patients:

- patients without diabetes mellitus
- patients with type 2 diabetes
- patients with diabetes of the exocrine pancreas (DEP)

Specific aims of the study are to clarify **1)** whether the reduction in insulin- and glucagon-positive islet areas, and therefore the size of the islets of Langerhans, is more pronounced in patients with DEP than in those without diabetes or with type 2 diabetes, **2)** whether islet size and insulin-positive area correlate with factors such as duration of diabetes, glucose-related parameters, tumour stage and grade, **3)** whether there is a specific distribution pattern of different diabetes types according to tumour stage and grade, and whether glycaemic control is affected by tumour size and malignancy grade.

2 Material and methods

2.1 Ethical vote

The study is based on the following vote of the Ethics Committee of the Medical Faculty of the Heinrich Heine University Düsseldorf: "Study to analyse gene expression patterns of pancreatic beta cells in patients after pancreatic surgery" dated 21 August 2012 (study number: 3923). The study is registered at ClinicalTrials.gov under NCT06150690.

2.2 Study collective

The study analysed data and samples from an existing biomaterial bank at the Institute for Clinical Diabetology at the German Diabetes Centre (DDZ) in Düsseldorf. A total of 179 patients were enrolled between July 2013 and January 2020. The biomaterial bank contains fasting blood samples, pancreatic tissue and abdominal adipose tissue from the study participants. The pancreatic tissue used in the study was only the tissue that had to be removed during the surgical procedure, i.e. for the current study no additional tissue was removed.

The study included patients over the age of 18 from the Department of General, Visceral and Paediatric Surgery at the University Hospital Düsseldorf, from the Department of General and Visceral Surgery, the Centre for Oncological and Minimally Invasive Surgery at the Florence Nightingale Hospital Düsseldorf and from the Department of Surgery at the Lukas Hospital Neuss. The patients were scheduled for partial or total pancreatectomy due to pancreatic neoplasia and gave written informed consent. Exclusion criteria for the study were patients who were minors and patients who had received neoadjuvant chemotherapy and/or radiotherapy with a direct effect on the pancreatic tissue.

After the patients were admitted as in-patients to the various recruitment sites, the DDZ was contacted by the doctors in charge of the participating centres. Detailed information was then provided to the patients by means of a signed informed consent form explaining the aims, methods, data handling, benefits and risks of the study.

2.3 Clinical patients´ data

Clinical patient data were collected using medical records and a specific case report form. Parameters such as age, sex, height, weight, known medical conditions and medications at the time of surgery were recorded. Particular attention was paid to the presence of known impairment of glucose metabolism (e.g. type 1 or type 2 diabetes mellitus) and the time of first diagnosis. Histopathological findings after resection were also recorded, including tumour stage and grade.

2.4 Fasting blood and clinical chemistry

Fasting blood samples were usually taken in the morning after at least eight hours of abstinence from food, nicotine and alcohol. A total of six tubes of blood were collected from each patient:

- Four 5 ml serum tubes
- One 3 ml EDTA tube
- One 1.3 ml tube for separate blood glucose analysis (see Table 7)

The serum and EDTA tubes were filled during a venous blood draw using a Safety-Lok blood collection set. For blood glucose measurement, a separate 1.3 ml Micro tube was filled with 1 ml of venous blood using a disposable syringe. All tubes were sequentially numbered.

The blood samples were then analysed in the Biomedical Analysis and Research Laboratory of the DDZ. The EDTA tube was placed on a roller mixer, while the four serum tubes were first centrifuged at 800 g for 10 minutes at 4 °C. Two of the four serum tubes were aliquoted into 3 ml cryovials each, one serum tube was divided into two 1.5 ml sample tubes and the other serum tube was divided into a 1.5 ml sample tube and a 2 ml glass tube (see Table 7). The aliquoted tubes were either analysed immediately or stored overnight in a refrigerator at 4 °C or in a freezer at -20 °C or -80 °C, respectively (see Table 9).

Clinical chemistry parameters were determined by laboratory staff applying quality assured laboratory methods. Regarding glucose metabolism, parameters such as HbA1c, fasting glucose, fasting insulin and fasting C-peptide were determined. Other parameters included bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, cholinesterase, lipase, amylase, pancreatic amylase, creatinine, free fatty acids, LDL cholesterol, total cholesterol, triglycerides and C-reactive protein (CRP).

Table 7: Materials for fasting blood analysis

EDTA tubes 3 ml BD Vacutainer	Becton Dickinson GmbH, Heidelberg, Germany
Micro tube 1.3 ml Fluoride + Heparin	SARSTEDT AG & Co. KG, Nümbrecht, Germany
Serum tube 5 ml with separating gel and coagulation activator BD Vacutainer SSTTM Advance tube	Becton Dickinson GmbH, Heidelberg, Germany

EDTA: Ethylenediaminetetraacetic acid; SST: serum-separating tube.

Table 8: Materials for aliquoting

Cryovial, 3 ml, free-standing, external thread	Biozym Scientific GmbH, Hessisch Oldendorf, Germany
epT.I.P.S. Standard, Eppendorf Quality TM, 50-1000 µl, 71 mm, blue, colourless tips	Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany
Glass tube 2 ml	Peter Oemen GmbH, Essen, Germany
Sample tube 1.5 ml	Biozym Scientific GmbH, Hessisch Oldendorf, Germany

Table 9: Laboratory equipment for analytics at DDZ

RM5 mixer assistant	Karl Hecht GmbH & Co. KG, Sondheim, Germany
Eppendorf Reference 2, single channel, variable, 100-1000 µl, blue	Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany
Liebherr Medi Line fridge	Liebherr-International Deutschland GmbH, Biberach an der Riß, Germany
Freezer Liebherr Profil Line -20 °C	Liebherr-International Deutschland GmbH, Biberach an der Riß, Germany
Centrifuge Rotixa 50 RS refrigerated	Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany

°C: degrees Celsius

2.5 Pancreas sample collection

After partial or total pancreatectomy performed at the collaborating centres, the surgeon first performed a macroscopic assessment of the removed pancreatic fragment.

From the area identified macroscopically as healthy pancreatic tissue, a piece approximately 5×5 mm in size was excised. This tissue fragment was then divided into three equal segments using a disposable surgical scalpel. Each segment was placed into a separate mould containing a thin layer of pre-hardened Tissue-Tek and subsequently embedded in Tissue-Tek. The moulds were labelled with the study participants' serial numbers to ensure accurate identification of the tissue. For further curing, the moulds were immersed in 2-methylbutane, which, in combination with dry ice, provides rapid cooling that prevents crystallization and allows for gentle freezing. Once the Tissue-Tek was fully cured, the moulds were transferred into dry transport containers cooled with liquid nitrogen at approximately -196°C. The specimens were then stored at -80°C at the DDZ until further analysis.

2.6 Preparation of pancreas sections on the cryostat

The cryostat was pre-cooled to a temperature of -30°C for at least one hour prior to use. Tissue-Tek was then applied bubble-free to sample holder and the sample block was placed with its largest surface facing upwards. After cooling, the Tissue-Tek solidifies within a few minutes, securing the sample to the holder. The sample holder was then mounted on the microtome, and the cutting blade was carefully placed in the blade holder. The microtome was manually advanced towards the blade, and the angle between the sample and blade was optimised using the dynamically adjustable joint.

Prior to thin sectioning, excess tissue was removed by rotating the blade to expose the pancreatic tissue. This process could be accelerated by using thicker sections (e.g., 40 µm). Once the pancreatic tissue was visible, thin sections of a defined thickness of 8 µm were prepared.

An anti-roll plate was placed on the sectioning table and the distance between the table and the plate was manually adjusted to prevent the sections from curling after sectioning. Turning the cutter wheel moved the microtome and specimen towards the blade within the defined cutting area. If the sections curled despite the anti-roll plate, a brush and tweezers were used to smooth them.

The sections were transferred to the top of a microscope slide placed on the cutting table. After sectioning, the sample holder was briefly thawed to release the sample block easily. The block was then completely removed from the slide with a razor blade, returned to its mould and re-frozen at -80°C.

After sectioning, the sections were air dried for approximately one hour and fixed in acetone for ten minutes, followed by another short air-drying period. The tissue sections were then circled with a grease pencil (Dako Pen), containing a fat-soluble, water-repellent solution. This solution helps to retain reagents during immunohistochemical staining for insulin and glucagon. Once the solution had dried, the sections were either stored at -80°C for later use or stained immediately (see Table 12; Table 13). The prepared thin sections of the pancreas corresponded to samples taken from the head, body, or tail of the organ. A total of 46 pancreas samples and 1810 islets of Langerhans were analysed in the study.

2.7 Immunohistochemical insulin and glucagon staining

If the tissue sections were frozen, they first had to be thawed and air dried for 20–30 minutes before processing. This step was omitted if processing was performed immediately after sectioning without freezing. The sections were then washed in 1x phosphate buffered saline (PBS) for 5 minutes, followed by blocking of endogenous peroxidase activity with 0.3% H_2O_2 in methanol

for 30 minutes. The sections were then washed three times for 2 minutes each with 1x PBS (see Table 11).

Detection of insulin and glucagon was performed using the Vector Laboratories VEC-TASTAIN ABC kit, which contained normal goat serum, a secondary antibody and reagents for the avidin-biotin (AB) complex (Table 10). Normal goat serum was diluted with 0.5% bovine serum albumin (BSA) in PBS, applied to the sections, and incubated for 20 minutes (see Table 11). Normal serum contains antibodies that bind to reactive sites on the tissue, minimising non-specific binding of primary and secondary antibodies. BSA blocks non-specific protein binding sites, ensuring specificity for antibody interactions. After blocking, the primary antibody specific for insulin (guinea pig anti-insulin antibody) or specific for glucagon (rabbit anti-glucagon antibody) was applied and the sections were incubated for 18 hours at room temperature in a humid environment to prevent drying. The sections were then washed three times for 2 minutes each with 1x PBS.

The secondary antibody (anti-guinea pig IgG antibody for insulin and anti-rabbit IgG for glucagon), which binds specifically to the primary antibody, is biotinylated and was applied for 30 minutes. The sections were then washed again (3 times for 2 minutes each with 1x PBS). Avidin and biotinylated horseradish peroxidase (HRP) were mixed and applied to the tissue for 30 minutes. Avidin, a tetrameric protein, binds biotin with high specificity and stability. It forms a complex by binding the biotinylated HRP and the biotinylated secondary antibody on the tissue. The HRP acts as a reporter enzyme (see Table 11).

Following another round of washing (3 times for 2 minutes each with 1x PBS), diaminobenzidine (DAB) was applied under the microscope. DAB acts as a substrate for HRP, resulting in staining where insulin was present. Blocking of endogenous peroxidases by preincubating the sections with H_2O_2 at the beginning of the staining procedure was essential to prevent background staining caused by non-specific DAB conversion. The DAB reaction was stopped by immersing the sections in demineralized water from the Milli-Q system (see Table 10 and Table 12).

Subsequently, the sections were counterstained with haematoxylin to enhance the visibility of basophilic (acidic) structures like cell nuclei. Excess haematoxylin was rinsed off under tap water for 10 minutes (see Table 10). To preserve the sections, the tissue was dehydrated through a graded series of ethanol (70%, 80% and 99% ethanol) for 5 minutes each. Following a 5-minute incubation in xylene to remove the alcohol, a drop of Entellan was applied as a mounting medium and a coverslip was placed on top to ensure no air bubbles were present. The Entellan was allowed to harden for approximately 24 hours, after which the sections could be stored without refrigeration (see Table 10 and Table 11).

Table 10: Reagents or the immunohistochemical detection of insulin and glucagon

2-Methylbutan ≥ 99%	Carl Roth, Karlsruhe, Germany
FLEX Polyclonal Guinea Pig Anti-Insulin (primary antibody for insulin)	Agilent, Santa Clara, CA, USA
FLEX Polyclonal Rabbit Anti-Glucagon (primary antibody for glucagon)	Agilent, Santa Clara, CA, USA
Acetone	VWR, Radnor, PA, USA
Bovine Serum Albumin (BSA)	SERVA, Heidelberg, Germany/New York, USA
Entellan	Merck, Darmstadt, Germany
Ethanol ≥ 99,5%	Merck, Darmstadt, Germany
Hämatoxylin	Carl Roth Karlsruhe, Germany
ImmEdge™ Pen	Vector Laboratories, Burlingame, CA, USA
Liquid 3,3´-Diaminobenzidine (DAB) Substrate+ Chromogen System	DAKO, Carpinteria, CA, USA
Methanol ≥ 99,9%	Carl Roth, Karlsruhe, Germany
Rotifair 10x PBS pH 7,4	Carl Roth, Karlsruhe, Germany
VECTASTAIN ABC Kit	Vector Laboratories, Burlingame, CA, USA
VECTASTAIN Goat Anti-Guinea Pig IgG Biotinylated Antibody (secondary antibody for insulin) (ABC Kit)	Vector Laboratories, Burlingame, CA, USA
VECTASTAIN Goat Anti-Rabbit IgG Biotinylated Antibody (secondary antibody for glucagon) (ABC Kit)	Vector Laboratories, Burlingame, CA, USA
VECTASTAIN Normal Goat Serum (ABC Kit)	Vector Laboratories, Burlingame, CA, USA
Hydrogen peroxide 30%	Carl Roth, Karlsruhe, Germany
Xylene (isomer) > 98%	Carl Roth, Karlsruhe, Germany

PBS: phosphate buffered saline.

Table 11: Solutions for immunohistochemical detection of insulin and glucagon

10x PBS: 10x PBS pH 7,4 + 1000 ml Milli-Q H_2O PBS pH 7,4 + 1000
1x PBS: 900 ml Milli-Q H_2 + 100 ml 10x PBS
0,5 % BSA-PBS: 20 ml 1x PBS + 0,1 g BSA
0,6 % hydrogen peroxide in methanol: 75 ml methanol + 450 µl hydrogen peroxide 30%
Normal serum: 1 ml 0,5% BSA-PBS + 15 µl normal goat serum
Primary antibody: 850 µl 0.5 % BSA-PBS + 150 primary antibody
Secondary antibody: 940 µl 1x PBS + 50 µl normal goat serum + 10 µl secondary antibody
Avidin-biotin complex (AB complex): 980 µl 1x PBS + 10 µl solution (avidin) + 10 µl solution B (biotinylated horseradish peroxidase)
3,3'-diaminobenzidine (DAB): 980 µl substrate buffer + 20 µl chromogen + H_2O_2
70% ethanol solution: 70 ml ethanol + 30 ml demineralised H_2O
80% ethanol solution: 80 ml ethanol + 20 ml demineralised H_2O

PBS: phosphate buffered saline; BSA: bovine serum albumin.

Table 12: Laboratory Equipment

-80 °C freezer	Thermo Fisher Scientific, Waltham, MA, USA
Fridge / freezer	Liebherr, Switzerland; Kirsch, Willstätt-Sand, Germany
Ice machine (AF 100)	Scotsman, Milano, Italy
Kyrostat CM 3050S	Leica Biosystems, Nußloch, Germany
Microscope (Axioplan)	Carl Zeiss, Oberkochen, Germany
Milli-Q System (IQ 7000)	Merck, Darmstadt, Germany
Minishaker MS1 (Vortex-shaker)	IKA, Staufen, Germany
Pipettes (Reference, Research)	Eppendorf, Hamburg, Germany
Pipetting aid Easypet	Eppendorf, Hamburg, Germany
Centrifuge (Biofuge Fresco)	Heraeus, Hanau, Germany
Microscope with camera (Nikon Eclipse Ti)	Nikon GmbH, Düsseldorf, Germany
Dry transport container DS2	VWR International, Radnor, PA, USA

°C: degree Celsius.

Table 13: Consumables for immunohistochemical detection of insulin and glucagon

Cover glass	Thermo Fisher Scientific, Waltham, MA, USA; Gerhard Menzel, Braunschweig, Germany
Dako Pen	Agilent, Santa Clara, CA, USA
Disposable pipettes (Falcon Transfer Pipette)	Corning, Corning, NY, USA
ImmEdgeTM Hydrophobic Barrier PAP Pen ("Dako Pen")	Vector Laboratories, USA
Low profile microtome blades (Leica 819)	Leica, Nußloch, Germany
Menzel Glasses Superfrost Plus (object carrier)	Thermo Fisher Scientific, Waltham, MA, USA; Gerhard Menzel, Braunschweig, Germany
Parafilm M	Bemis, Neenah, WI, USA
Peel-A-Way Disposable Embedding Molds	Polysciences, Warrington, PA, USA; Sarstedt, Nümbrecht; Greiner Bio-One, Kremsmünster, Germany
Razor blades	Apollo, Solingen, Germany
Reaction tubes („Eppendorf tube") 2,0-1,5 µl	Sarstedt, Nümbrecht, Germany
Pipette tips 1000 + 200 + 10 µl	Sarstedt, Nümbrecht, Germany; Greiner Bio-One, Kremsmünster, Germany
Tissue-Tek O.C.T. Compound	Sakura Finetek, Alphen aan den Rijn, Netherlands
Disposable surgical scalpels	B. Braun, Melsungen, Germany

2.8 Microscopical analysis of stained thin sections

The sections were analysed in a blinded manner. Two tissue sections were evaluated per patient: one for insulin staining and the other for glucagon staining. Analysis of the stained sections was performed using a Nikon Eclipse microscope, an Intuos Pro Paper Edition L pen tablet, and Olympus cellSens Dimension software.

Initially, the tissue was scanned to locate the islets of Langerhans, identified by insulin or glucagon staining. Each identified islet was photographed at 20x magnification, and the images were used to manually measure the area by tracing the islet's perimeter. To determine the proportion of insulin-positive or glucagon-positive regions, the staining intensity was manually defined. A threshold value encompassing a specific range of colour tone, intensity, and saturation was estab-

lished. Within a section, all islets were analysed using the same settings. However, minor adjustments were made to reduce day-to-day variations in the staining characteristics parameters of the analysis. This manual normalization process ensured a high level of comparability across all sections. All results of the microscopical evaluation were expressed as μm^2.

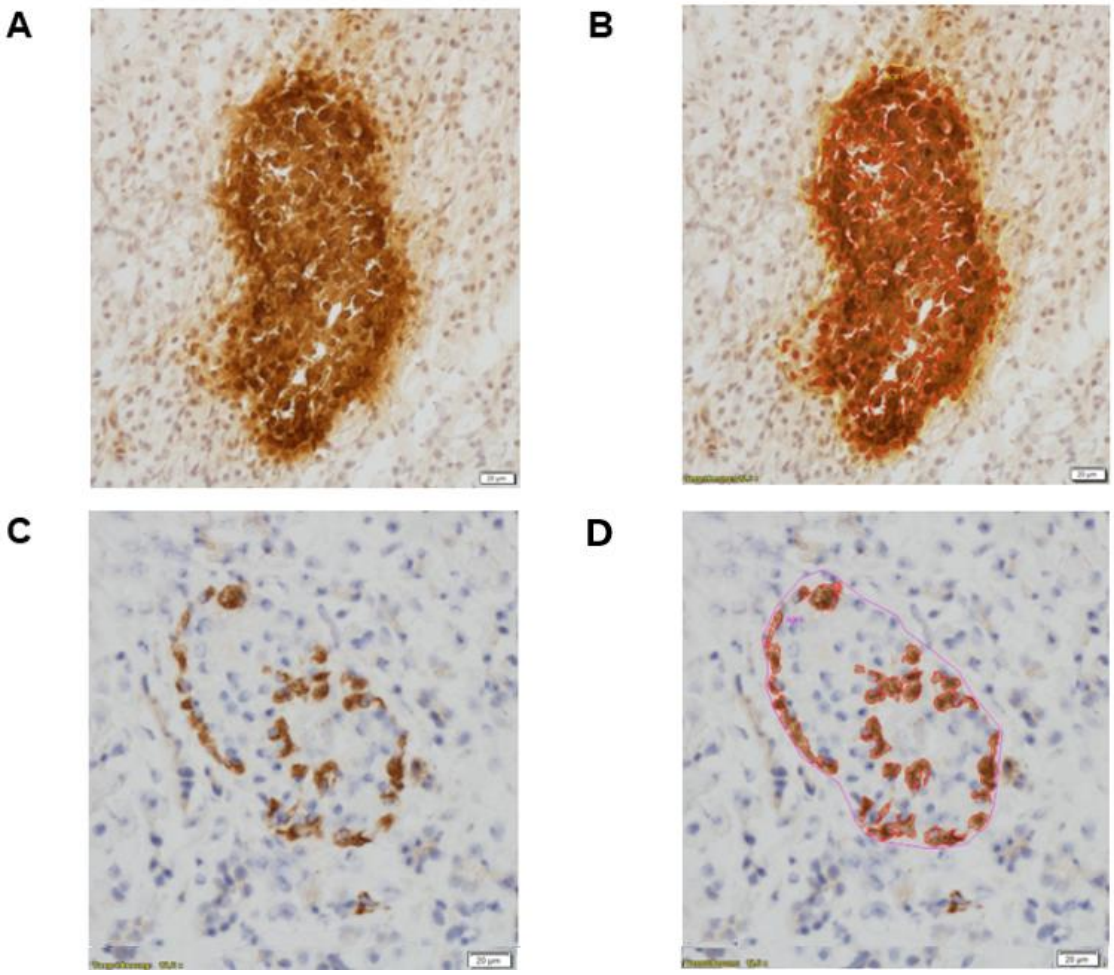

Fig. 1: Insulin and glucagon staining.

The figure presents microscopic images of pancreatic tissue at 20x magnification. Figure 1A and 1B show a pancreatic islet stained with Haematoxylin-Eosin and DAB, which colours insulin-producing cells brown (1A; 1B). Images 1C and 1D show glucagon-producing cells stained with Haematoxylin-Eosin and DAB, which colours glucagon-producing cells brown (1C; 1D). Fig. 1B and 1D display the islet area, with insulin-positive (1B) and glucagon-positive (1D) areas measured using Olympus cellSens Dimension software. The bar at the bottom of each figure represents 20 µm.

2.9 Homeostatic Model Assessment (HOMA)

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Beta Cell Function (HOMA-B) is a mathematical model used to estimate endogenous insulin resistance and beta cell function (Festa et al. 2008; Wallace et al. 2004). It is based on Turner's homeostasis model and is calculated using fasting glucose and fasting insulin levels (Turner et al. 1990). The model is based

on the feedback mechanism between the liver and pancreatic beta cells that regulates the balance between insulin secretion and hepatic glucose release. This allows a theoretical calculation of steady-state insulin and glucose levels. HOMA-IR and HOMA-B were calculated for each patient in the study. The formulae for these calculations are as follows:

- HOMA-IR= Fasting plasma insulin [mU/l] ∗ fasting plasma glucose [mmol/l]/ 22.5
- HOMA-B = 20 x fasting insulin [mU/l] / (fasting plasma glucose [mmol/l] - 3.5)

HOMA-IR values above 1.85 in women and 2.17 in men are likely to indicate insulin resistance (Gayoso-Diz et al. 2013). For simplicity in clinical practice, insulin resistance is considered unlikely with a HOMA-IR below 2. In the current study, insulin resistance was defined as a HOMA-IR above 2. Normal values for HOMA-B are reported differently in the literature. In this study, a normal HOMA-B was considered to be between 80-100%. In addition, a more advanced algorithm, HOMA2-IR and HOMA2-B, was developed. This improved model takes into account the non-linear relationship between glucose and insulin and incorporates variability in hepatic and peripheral glucose resistance, providing a more accurate assessment (Fan et al. 2022; Song et al. 2016).

2.10 Classification of diabetes mellitus

In this study, participants were categorized into three groups based on the evidence-based guidelines from the German Diabetes Association (DDG):

- Patients without diabetes mellitus (ND)
- Patients with type 2 diabetes mellitus (T2D)
- Patients with diabetes of the exocrine pancreas (DEP).

Currently, there are no standardized diagnostic criteria for DEP. Therefore, this study followed the criteria proposed by Hart et al. (2016). According to Hart et al., DEP is diagnosed when the following conditions are met:

- presence of diabetes mellitus
- presence of an exocrine pancreatic disease, such as acute or chronic pancreatitis or pancreatic neoplasia
- temporal relationship between the initial diagnosis of exocrine pancreatic disease and the onset of diabetes mellitus (Hart et al. 2016).

To further exclude the possibility of type 1 diabetes mellitus in the patient cohort, type 1 diabetes autoantibodies, including ICA, GAD65, IAA, IA-2, IA-2beta and ZnT8 autoantibodies, were tested for each patient at the DDZ. One patient in the cohort was tested positive for GAD 65 autoantibodies and was subsequently excluded from the study.

Table 14 shows the criteria used to categorise patients into the three groups.

Table 14: Classification of diabetes mellitus in the present work

Patients without diabetes (ND)	• FPG <100 mg/dl (or < 5.6 mmol/l) • 2h-PG oGTT <140 mg/dl (or 7.8 mmol/l). • HbA1c < 6.5% (< 48 mmol/mol Hb)
Patients with type 2 diabetes mellitus (T2D)	• FPG of ≥ 126 mg/dl (or ≥ 7.0 mmol/l) or • PG value of ≥ 200 mg/dl (or ≥ 11.1 mmol/l) after 2 hours during oGTT • HbA1c > 6.5% (> 48 mmol/mol Hb) • diabetes mellitus diagnosed more than 12 months before presentation
Patients with diabetes of the exocrine pancreas (DEP)	• FPG of ≥ 126 mg/dl (or ≥ 7.0 mmol/l) or • PG value of ≥ 200 mg/dl (or ≥ 11.1 mmol/l) after 2 hours during oGTT • HbA1c > 6.5% (> 48 mmol/mol Hb) • diabetes mellitus diagnosed less than 12 months before presentation

FPG: fasting plasma glucose; PG: plasma glucose; OGTT: oral glucose tolerance test; HbA1c: Haemoglobin A1c

2.11 Classification of the pancreatic tumour

Histopathological evaluation of tissue samples obtained from partial or total pancreatectomy was performed by the pathology institutes associated with the respective hospitals:

- University Hospital Düsseldorf (UKD): Institute of Pathology, UKD
- Florence Nightingale Hospital Düsseldorf-Kaiserswerth (FNK): Institute of Pathology, Bethesda Hospital Duisburg
- Lukas Hospital Neuss: Institute of Pathology, Lukas Hospital Neuss

The tissue samples were analysed based on the most recent (8th edition) internationally standardized criteria set by the UICC (Bertero et al. 2018) and the American Joint Committee on Cancer (AJCC). Notably, the 8th edition of the TNM classification of malignant tumours was published in 2017. Histopathological findings from samples classified prior to 2017 followed the 7th edition from 2009, which introduced certain changes to the T and N classifications. For example, in the 2009 edition, a primary tumour classified as T3 was defined by extrapancreatic spread without involvement of the coeliac trunk or the superior mesenteric artery.

However, in the 8th edition, T3 is defined by tumour size, specifically when it exceeds 4 cm. Furthermore, the 2009 N classification distinguished only two categories: N0 (no regional lymph node metastases) and N1 (regional lymph node metastases). The 8th edition introduces a third category, N2, for cases with more than 3 regional lymph nodes involved, whereas N1 is used for 1-3 regional lymph nodes involved. These updates were incorporated into the analysis of the current study, and the histopathological results were adjusted according to the 8th edition of the TNM classification (Seufferlein et al. 2024).

The TNM classification, corresponding tumour stages (see Table 5) and tumour grading (see Table 15) were documented from the histopathology reports and assigned to each patient. Tumour grading reflects the degree of malignancy and indicates the extent to which the tumour tissue has deviated from its original benign state.

Table 15: Tumour grading

TUMOUR GRADE	DEFINITION
G1	Well-differentiated malignant tissue
G2	Moderately differentiated malignant tissue
G3	Poorly differentiated malignant tissue

The patient cohort was categorised as follows:

- Non-malignant tumours
- G1 tumours
- G2 tumours
- G3 tumours

The non-malignant (NM) group included patients diagnosed with cystic, non-malignant precancerous lesions. These included intraductal papillary mucinous neoplasms (IPMNs) with low-grade dysplasia (n=8) and serous cystadenomas (n=1) identified on histopathological examination. In addition, one patient had no evidence of malignant cellular changes or associated benign pancreatic lesions. A total of 10 patients were classified as having NM tumours.

Patients were further stratified by tumour stage as follows:

- Stage IA/IB
- Stage IIA/IIB
- Stage III/IV

For statistical evaluation, tumour stages were combined into the following three groups IA and IB, IIA and IIB, and III and IV.

2.12 Statistical analysis

Statistical analyses and graph generation were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean ± standard error of the mean (SEM) as well as ± standard deviation (SD). P-values less than 0.05 were considered statistically significant, with significance levels set at $p < 0.05$, $p < 0.01$ and $p < 0.001$. An unpaired t-test was used for comparisons between two groups. Differences in percentage distributions were assessed using Fisher's exact test. One-way analysis of variance (ANOVA) was used for comparisons between more than two groups. The Chi^2 test was used to assess differences in group distributions. Linear correlations were analysed using Pearson's correlation, with the correlation coefficient (r) indicating the strength and direction of the relationship. Statistically significant outliers were excluded from the analysis. Decimals for clinical parameters have been rounded to one decimal place, while the correlation coefficient has been rounded to two decimal places.

3 Results

3.1 Patients´ characteristics

The study included a total number of 46 patients: 28 patients without diabetes (eleven males/17 females), seven with type 2 diabetes mellitus (one male/six females) and eleven with diabetes of the exocrine pancreas (two males/nine females). Table 15 shows the distribution of the clinical characteristics of the patients in the three different groups ND, T2D and DEP.

Table 16: Patients´ characteristics

	ND	**T2D**	**DEP**
Total number (male)	28 (11)	7 (1)	11 (2)
Age (years)	68 ± 10	70 ± 14	69 ± 11
Diabetes duration (months)	-	47 ± 26	4 ± 3 ###
Duration of pancreatic disease (months)	7 ± 21	23 ± 47	2 ± 2
BMI (kg/m²)	25.5 ± 3.6	30.4 ± 4.4*	26.0 ± 4.5
Fasting plasma glucose [mg/dl]	88 ± 14	162 ± 38***	140 ± 37 ***
Fasting plasma glucose [mmol/l]	4.9 ± 0.8	9.0 ± 2.1***	7.8 ± 2.1***
HbA1c [%]	5.6 ± 0.4	8.0 ± 1.2***	7.0 ± 1.1***
HbA1c [mmol/mol **Hb**]	38 ± 5	64 ± 13***	53 ± 12***
Fasting plasma insulin [µU/ml]	10.8 ± 13.0	13.8 ± 7.1	20.4 ± 24.0
C-peptide [nmol/l]	8.91 ± 9.63	8.92 ± 3.82	6.64 ± 6.40
CRP [mg/dl]	3.8 ± 7.7	0.5 ± 0.4	0.5 ± 0.8
Bilirubin [mg/dl]	1.4 ± 3.0	0.5 ± 0.1	0.6 ± 0.2
Lipase [U/l]	106 ± 236	51 ± 46	62 ± 73
Amylase [U/l]	82.9 ± 101.8	52.7 ± 23.9	52.5 ± 26.9
Pancreatic amylase [U/l]	61.7 ± 103.1	28.8 ± 21.2	26.9 ± 23.7
Number of patients on insulin treatment	-	4	8

ND: patients without diabetes mellitus; T2D: patients with type 2 diabetes mellitus; DEP: patients with diabetes of the exocrine pancreas; BMI: Body Mass Index; HbA1c: Haemoglobin A1c; CRP: C-Reactive Protein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HOMA-B: Homeostatic Model Assessment of Beta Cell Function. Mean, standard error of the mean (SEM) and statistical significance (* $p < 0.05$ compared to ND, ** $p < 0.01$ compared to ND, *** $p < 0.001$ compared to ND; ### $p < 0.001$ compared to T2D) are indicated. One-way analysis of variance (ANOVA) was used for testing.

There was no difference in the age distribution within the three groups. Patients with T2D had a higher BMI than those with ND (30.4 ± 4.4 kg/m² *vs.* 25.5 ± 3.6 kg/m²; $p < 0.05$). There was no difference in BMI between T2D and DEP as well as between DEP and ND.

A difference in the duration of diabetes was observed between patients with T2D and those with DEP. The average duration of diabetes was shorter in patients with DEP (4 ± 3 months) compared to those with T2D (47 ± 26 months), $p < 0.001$.

There was no difference in the duration of pancreatic disease between the three groups. Fasting plasma glucose levels were higher in patients with T2D (162 ± 38 mg/dl), and DEP (140 ± 37 mg/dl) compared to ND persons (88 ± 14 mg/dl), both $p < 0.001$. Fasting plasma glucose levels were comparable in T2D and DEP patients.

The same trend was observed for HbA1c in the three groups. HbA1c values were higher in T2D (8.0 ± 1.2 %) and DEP (7.0 ± 1.1 %) than in ND (5.6 ± 0.4 %) persons (both $p < 0.001$). HbA1c was not different between T2D and DEP subjects. There were no differences in fasting plasma insulin, HOMA-B, C-peptide, CRP, lipase, amylase and pancreatic amylase levels between the three groups.

3.2 HOMA-IR and HOMA-B in the patients' groups

HOMA-IR values were higher in the DEP (6.1 ± 7.0) and T2D (4.7 ± 2.7) groups compared to the ND group (2.1 ± 2.4) (both $p < 0.05$). There was no difference in HOMA-IR levels between patients with T2D and DEP (Fig. 2 A).

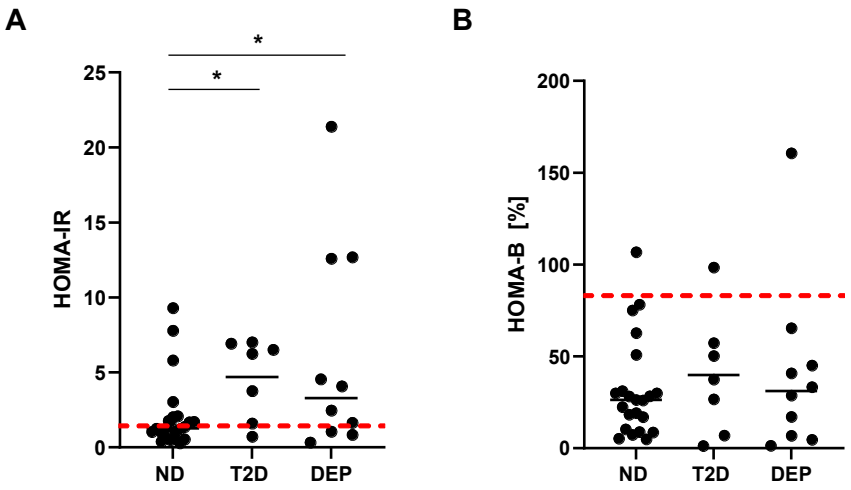

Fig. 2: HOMA-IR and HOMA-B in the patients' groups

ND: patients without diabetes mellitus; T2D: patients with type 2 diabetes mellitus; DEP: patients with diabetes of the exocrine pancreas; HOMA-IR: Homeostasis Model Assessment for insulin resistance; HOMA-B: Homeostasis Model Assessment for beta cell function. Mean (indicated by black horizontal line) and statistical significance (*$p < 0,05$ compared to ND) are indicated. **A:** Scatterplot of HOMA-IR in patients with ND, T2D and DEP. **B:** Scatterplot of HOMA-B in patients with ND, T2D and DEP. The red dashed line in Fig. 2A indicates the upper limit of the normal range for HOMA-IR (= 2). The red dashed line in Fig. 2B indicates the lower limit of the normal range for HOMA-B (= 80%).

In a sub-analysis between patients with impaired fasting glucose (IFG) and normal fasting glucose (NFG), seven patients had IFG, defined as FPG in the range of 100-125 mg/dl. The IFG group had higher mean HOMA-IR levels (6.0 ± 3.6) than the NFG group (1.9 ± 2.9) ($p < 0.01$). No difference in HOMA-IR was observed between IFG, T2D and DEP.

No difference in HOMA-B was found between the ND, T2D and DEP groups. The measured mean HOMA-B values were 31.8 ± 27.0 in the ND group, 39.9 ± 33.1 in the T2D group and 40.5 ± 46.8 in the DEP group.

In a sub-analysis between seven patients with IFG and 21 with NFG, no difference in mean HOMA-B was observed. There was also no difference in mean HOMA-B between the IFG group and the T2D and DEP groups.

3.3 Correlation between HOMA-IR and HOMA-B with diabetes duration and duration of pancreatic disease

Table 17: Correlation between HOMA-IR and HOMA-B with diabetes duration and pancreatic disease onset diagnosis

Correlation	r	p
HOMA-IR | Diabetes duration	-0.012	0.905
HOMA-IR | Duration of PD	-0.093	0.446
HOMA-B | Diabetes duration	-0.079	0.732
HOMA-B | Duration of PD	-0.256	0.042

HOMA-IR: Homeostasis Model Assessment for insulin resistance; HOMA-B: Homeostasis Model Assessment for beta cell function; PD: pancreatic disease; r: Pearson correlation coefficient and p: statistical significance is indicated. Simple Linear Regression was used for testing.

In a further analysis, the correlation between HOMA-IR and HOMA-B and the duration of diabetes and pancreatic disease was determined. As shown n in Table 16, no correlation was found between HOMA-IR and duration of diabetes as well as duration of pancreatic disease. Moreover, no correlation was found between HOMA-B and duration of diabetes. However, HOMA-B correlated negatively with the period since the diagnosis of pancreatic disease ($r= -0.256$, $p < 0.05$).

3.4 Morphological characteristics of pancreatic islets in the patients' groups

Morphological characteristics of pancreatic islets in the patients´ group were determined microscopically and the size of islets and their insulin- and glucagon-positive areas were quantified.

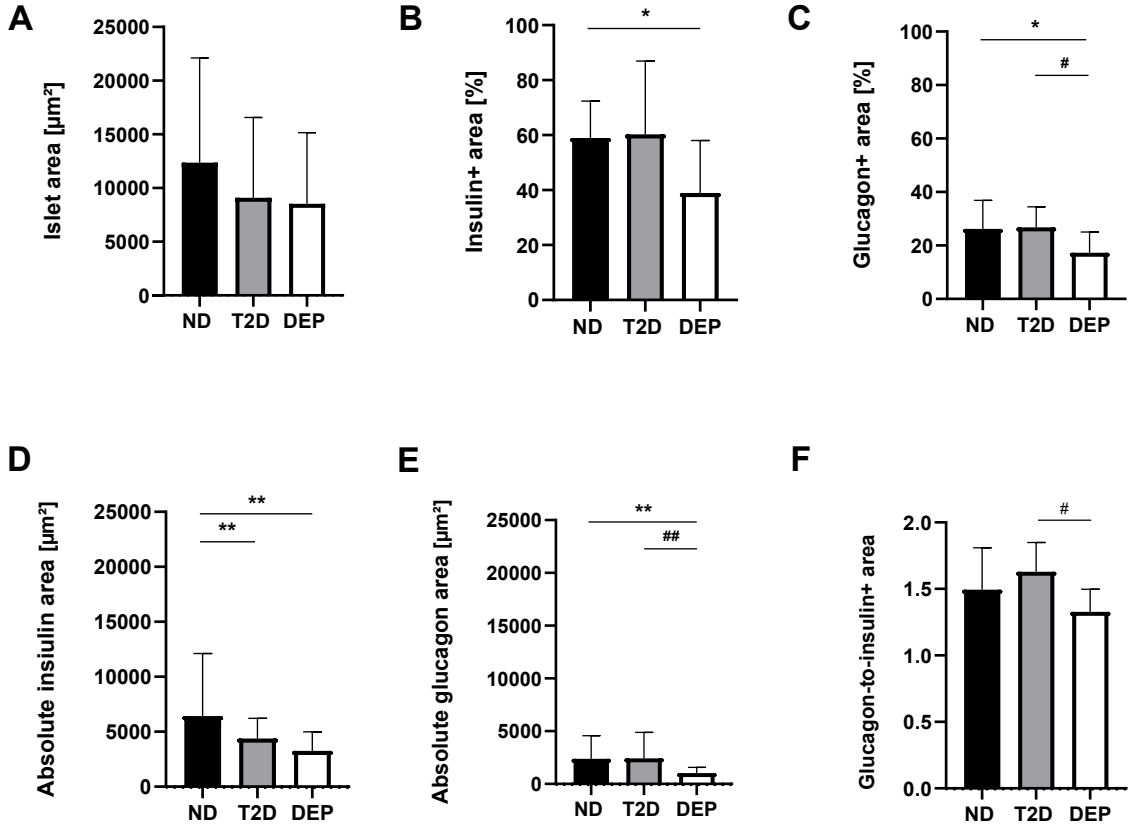

Fig. 3: Morphological characteristics of pancreatic islets in the patients' groups

ND: patients without diabetes mellitus; T2D: patients with type 2 diabetes mellitus; DEP: patients with diabetes of the exocrine pancreas. Mean, standard deviation and statistical significance (* $p < 0.05$ compared to ND, ** $p < 0.01$ compared to ND; # $p < 0.05$ compared to T2D; ## $p < 0.01$ compared to T2D) are indicated. **A:** Area (µm²) of the islets of Langerhans in the three groups. **B:** Area of the percentual insulin-positive area in the three groups **C:** Area of the percentual glucagon-positive area in the three groups. **D:** Area of absolute insulin-positive area the three groups. **E:** Area of absolute glucagon-positive area in the three groups. **F:** Ratio of glucagon- to insulin-positive area in the three groups. One-way analysis of variance (ANOVA) was used for testing.

While no significant differences were observed in the size of islets of Langerhans among the three groups (Fig. 3A), the percentual insulin-positive islet area was lower in DEP (39.0% ± 19.0%) compared to ND (58.9% ± 13.4%) with $p < 0.05$ (Fig. 3B). No difference was observed between the percentual insulin-positive area in ND (58.9% ± 13.4%) and T2D (60.3% ± 26.7%, Fig. 3B). The percentual glucagon-positive islet area was lower in DEP (17.3% ± 7.7%) compared to both, T2D (26.8% ± 7.7%) and ND (26.2% ± 10.7%), both $p < 0.05$ (Fig. 3C). However, no difference in the glucagon-positive area was observed between ND (26.2% ± 10.7%) and T2D (26.8% ± 7.7%; Fig. 3C) ($p > 0.05$).

The absolute insulin-positive area was smaller in T2D (4,389.1 µm² ± 1,819.4 µm²), and DEP (3,259.7 µm² ± 1736.1 µm²) compared to ND (6,425.0 µm² ± 5,686.4 µm²), both $p < 0.01$ (Fig. 3D). The absolute glucagon-positive area was significantly smaller in DEP (1,024.6 µm² ± 547.5 µm²) compared to both T2D (2,437.6 µm² ± 2,448.0 µm²) and ND (2,382.7 µm² ± 2,198.6 µm²), both $p < 0.01$ (Fig. 3E). The ratio of glucagon-to-insulin-positive area was found to be lower in DEP (1.3 ± 0.2) compared to T2D (1.6 ± 0.3), $p < 0.05$ (Fig. 3F). No significant differences in the ratio of glucagon-to-insulin-positive areas were observed between T2D (1.6 ± 0.3) and ND (1.5 ± 0.8), or between DEP (1.3 ± 0.2) and ND (1.5 ± 0.8), both $p > 0.05$.

3.5 Correlation of diabetes duration and morphological characteristics of pancreatic islets in the patients' groups

In both T2D and DEP, the size of the islets of Langerhans area negatively correlated with diabetes duration ($r = -0.392$, $p < 0.001$ and $r = -0.233$, $p < 0.05$, respectively; Figg. 4A; 4B). In T2D, the percentual insulin-positive area showed a positive correlation, while the absolute insulin-positive area showed a negative correlation with diabetes duration ($r = 0.447$, $p < 0.001$ and $r = -0.219$, $p < 0.01$, respectively; Fig. 4C). In DEP, both the percentual and the absolute insulin-positive area showed a negative correlation with diabetes duration ($r = -0.551$, $p < 0.001$ and $r = -0.394$, $p < 0.001$, respectively; Fig. 4D). In addition, the percentual and the absolute glucagon-positive areas in T2D negatively correlated with diabetes duration ($r = -0.259$, $p < 0,01$ and $r = -0.444$, $p < 0.001$, respectively; Fig. 4E). No correlation was found between the percentual glucagon-positive area in DEP and the duration of diabetes (Fig. 4F). A negative correlation was observed between the absolute glucagon-positive area and the duration of diabetes in DEP ($r = -0.310$, $p < 0.01$; Fig. 4F).

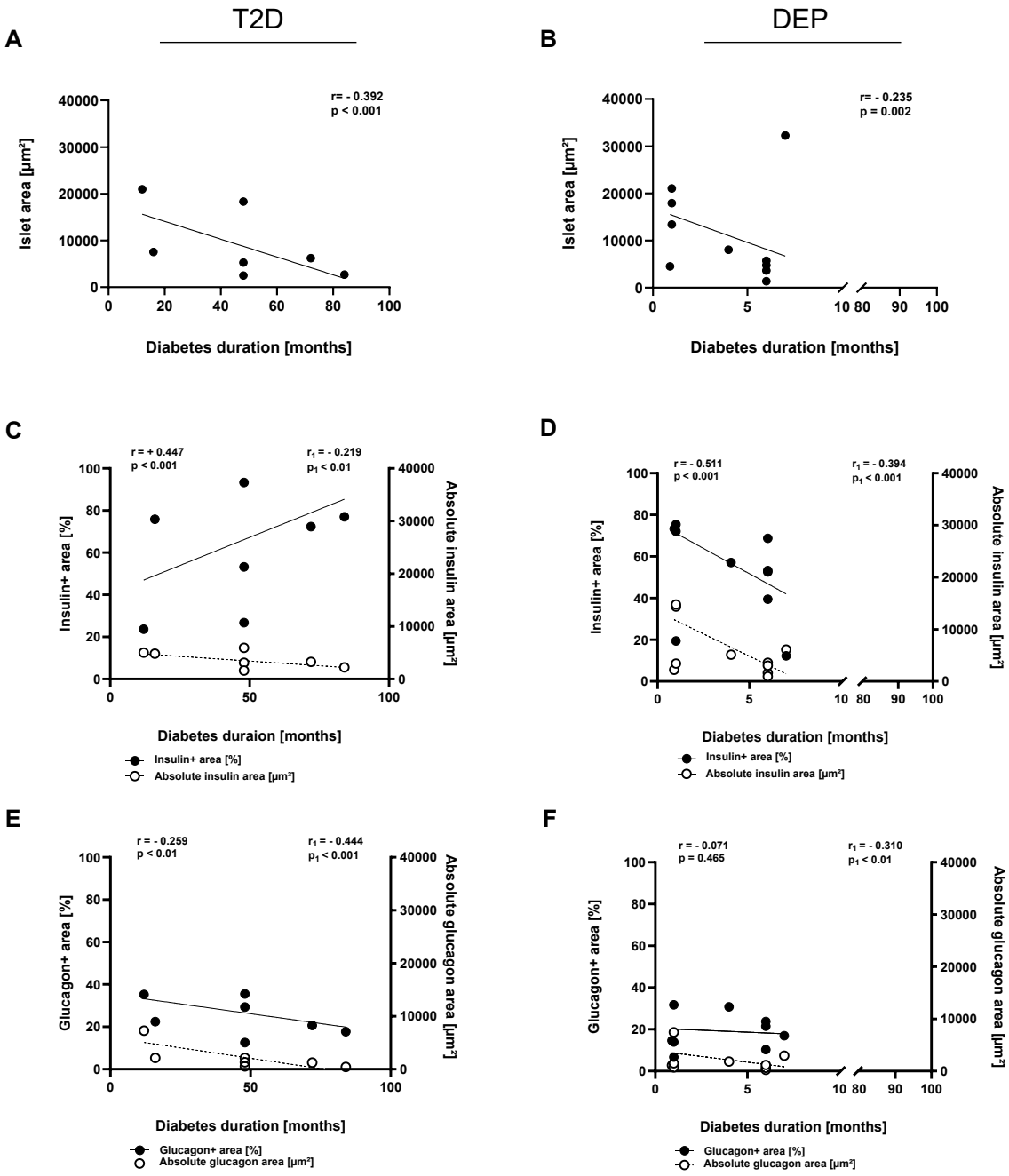

Fig. 4: Correlation of diabetes duration and morphological characteristics of pancreatic islets in the patients' groups

T2D: patients with type 2 diabetes mellitus; DEP: patients with diabetes of the exocrine pancreas; r: Pearson correlation coefficient and p: statistical significance is indicated. **A/B:** Correlation between diabetes duration and area of the islets of Langerhans (µm²) in T2D (A) and DEP (B). **C/D:** Correlation between diabetes duration and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²) in T2D (C) and DEP (D). **E/F:** Correlation between diabetes duration and percentual glucagon-positive area (%) as well as absolute glucagon-positive area (µm²) in T2D (E) and DEP (F).

3.6 Correlation of BMI and morphological characteristics of pancreatic islets in the patients' groups

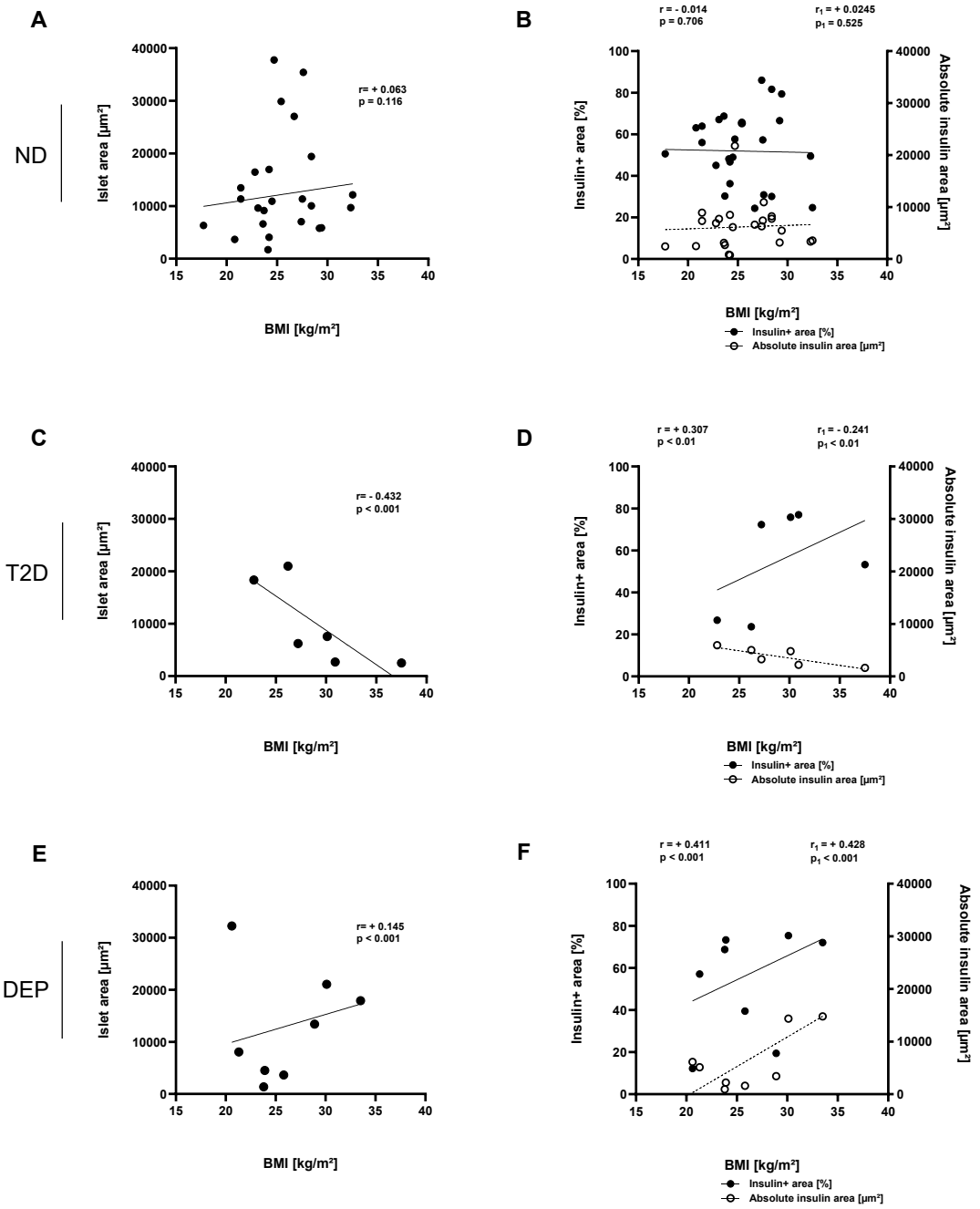

Fig. 5: Correlation of BMI and morphological characteristics of pancreatic islets in the patients' groups

ND: Patients without diabetes mellitus; T2D: Patients with type 2 diabetes mellitus; DEP: Patients with diabetes of the exocrine pancreas; BMI: Body Mass Index; r: Pearson correlation coefficient and p: statistical significance are indicated. **A/B:** Correlation between BMI and area of the islets of Langerhans (µm²; A) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; B) in ND (n= 24). **C/D:** Correlation between BMI and islets of Langerhans area (µm²; C) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; D) in T2D (n= 6). **E/F:** Correlation between BMI and area of the islets of Langerhans (µm²; E) and percentual insulin-positive area (%; F) as well as absolute insulin-positive area (µm²) in DEP (n= 8).

In ND, neither islet size nor insulin-positive islet area correlated with BMI (Figg. 5A; 5B).

In patients with T2D, BMI and islet size were negatively correlated ($r = -0.432$, $p < 0.001$) (Fig. 5C). BMI showed a positive correlation with the percentual insulin-positive area ($r = +0.307$, $p < 0.01$; Fig. 5D). In contrast, BMI and absolute insulin-positive area in T2D were negatively correlated ($r = -0.241$, $p < 0.01$; Fig. 5D).

The DEP cohort showed a positive correlation between BMI and islet size ($r = +0.145$, $p < 0.001$; Fig. 5E). A positive correlation was also observed between BMI and both, percentual and absolute insulin-positive area ($r = +0.411$, $p < 0.001$ and $r = +0.428$, $p < 0.001$, respectively; Fig. 5F).

3.7 Correlation between fasting plasma glucose levels and morphological characteristics of pancreatic islets in the patients' group

No correlation was found between size of the islets of Langerhans area and fasting glucose levels (6A). However, a negative correlation was observed between both the percentual insulin-positive area and the absolute insulin-positive area with fasting glucose levels ($r = -0.205$, $p < 0{,}001$ and $r = -0.084$, $p < 0.05$, respectively; Fig. 6B).

In the T2D cohort, a negative correlation was identified between islet size and fasting glucose levels ($r = -0.063$, $p < 0.05$; Fig. 6C). In addition, percentual and absolute insulin-positive areas were negatively correlated with fasting glucose levels ($r = -0.152$, $p < 0.05$ and $r = -0.170$, $p < 0.05$, respectively; Fig. 6D).

In the DEP cohort, a negative correlation was observed between the size of the islets of Langerhans area and fasting glucose levels ($r = -0.217$, $p < 0.05$; Fig. 6E). The percentual insulin-positive area was positively correlated with fasting glucose levels, while the absolute insulin-positive area was negatively correlated ($r = +0.158$, $p < 0.05$ and $r = -0.295$, $p < 0.001$, respectively; Fig. 6F).

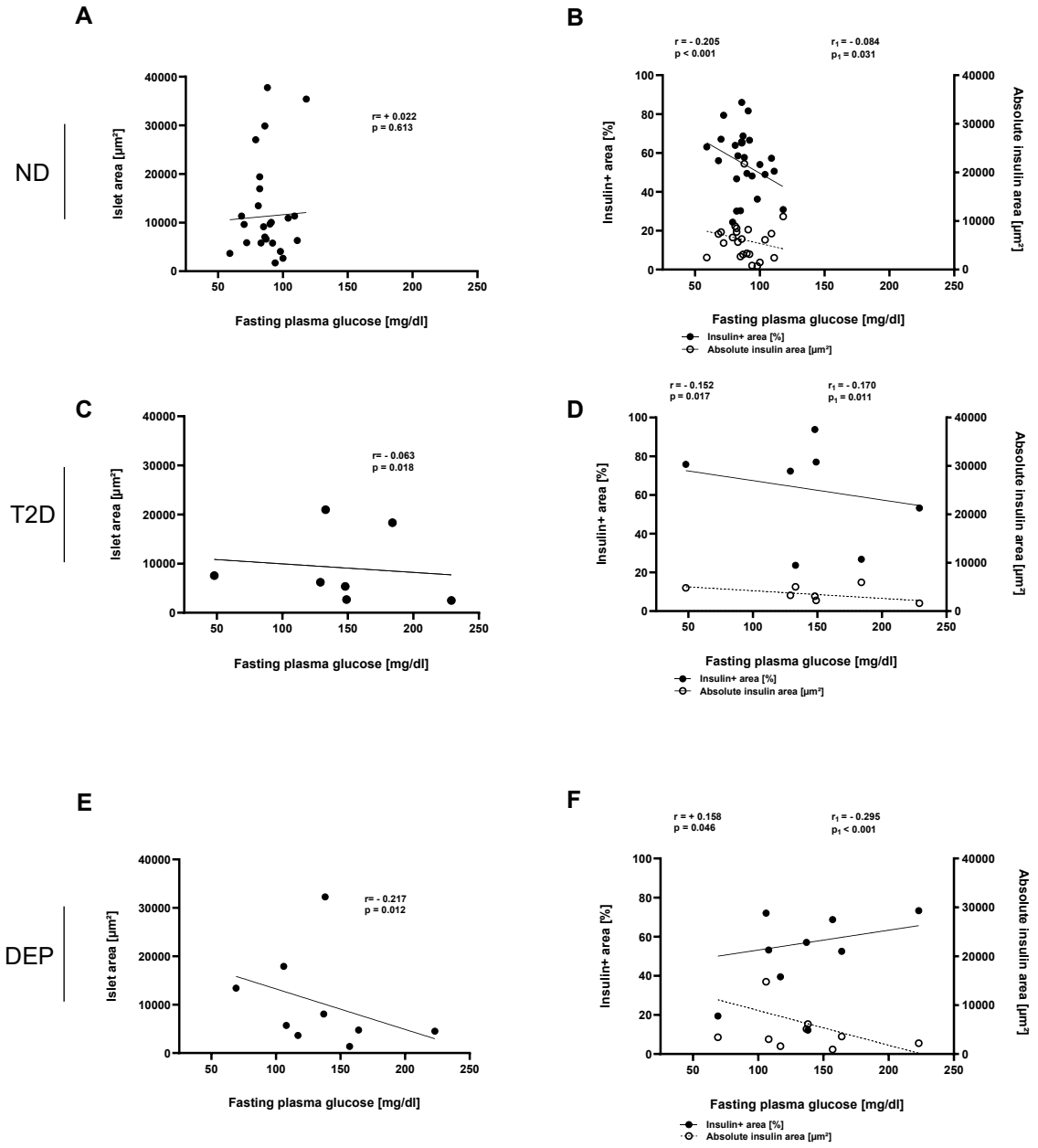

Fig. 6: Correlation of fasting plasma glucose levels and morphological characteristics of pancreatic islets in the patients' groups

ND: Patients with no diabetes mellitus; T2D: Patients with type 2 diabetes mellitus; DEP: Patients with diabetes of the exocrine pancreas; r: Pearson correlation coefficient and p: statistical significance are indicated. **A/B:** Correlation between fasting plasma glucose and area of the islets of Langerhans (µm²; A) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; B) in ND (n= 25). **C/D:** Correlation between fasting plasma glucose and area of the islets of Langerhans (µm²; C) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; D) in T2D (n= 7). **E/F:** Correlation between fasting plasma glucose and area of the islets of Langerhans (µm²; E) in DEP and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; F) in DEP (n= 9). Simple Linear Regression was used for testing.

3.8 Correlation between HbA1c and morphological characteristics of pancreatic islets in the patients' groups

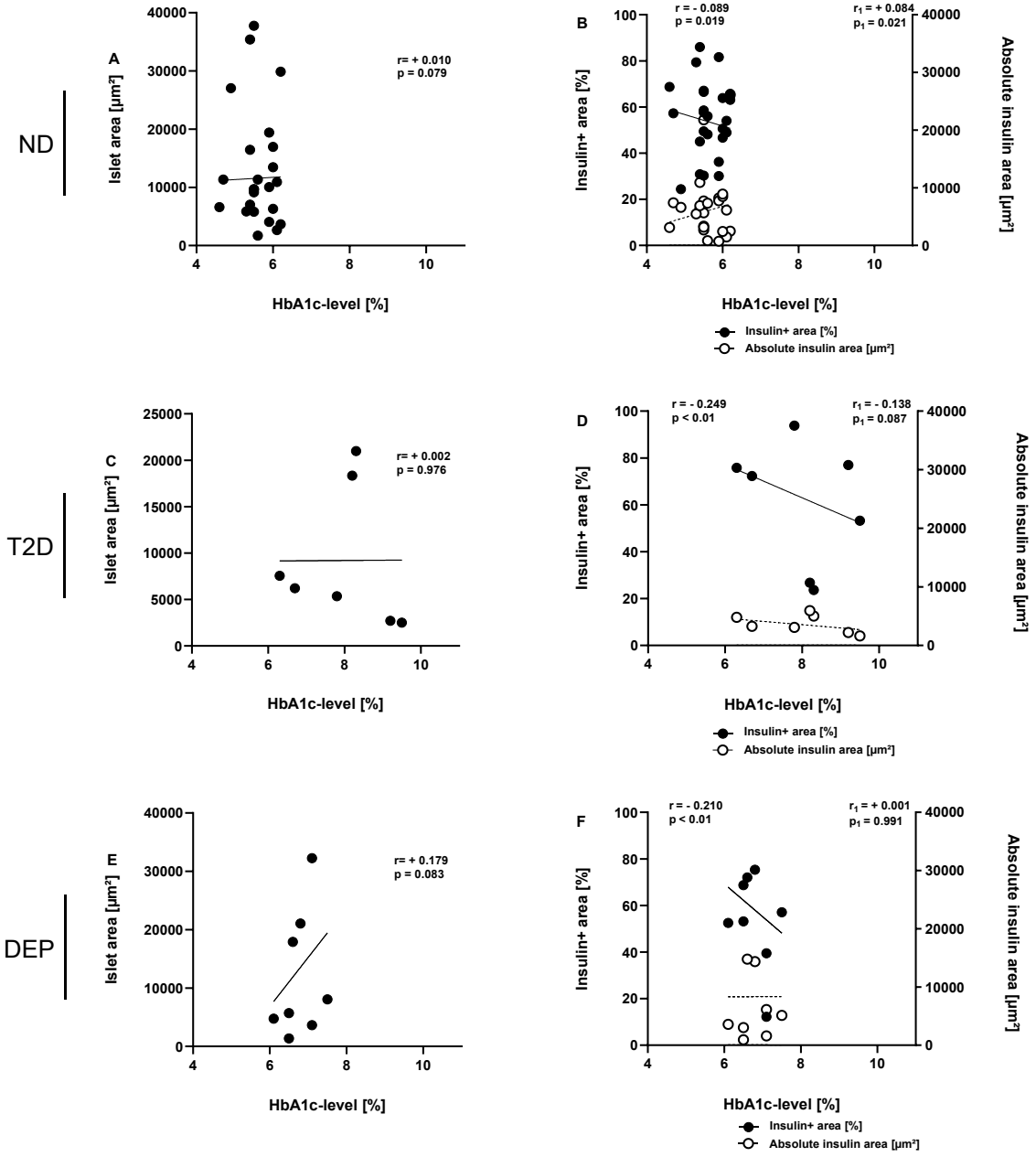

Fig. 7: Correlation of HbA1c-levels and morphological characteristics of pancreatic islets in the patients' groups

ND: Patients with no diabetes mellitus; T2D: Patients with type 2 diabetes mellitus; DEP: Patients with diabetes of the exocrine pancreas; HbA1c: Haemoglobin A1c; r: Pearson correlation coefficient and p: statistical significance are indicated. **A/B:** Correlation between HbA1c and area of the islets of Langerhans (µm²; A) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; B) in ND (n= 25). **C/D:** Correlation between HbA1c and area of the islets of Langerhans (µm²; C) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; D) in T2D (n= 7). **E/F:** Correlation between HbA1c and area of the islets of Langerhans (µm²; E) and percentual insulin-positive area (%) as well as absolute insulin-positive area (µm²; F) in DEP (n= 8). Simple Linear Regression was used for testing.

There was no correlation between the size of the islets of Langerhans area and HbA1c in the ND group (Fig. 7A). However, a negative correlation was observed between the percentual insulin-positive area and HbA1c ($r = -0.089$, $p < 0.05$; Fig. 7B). In contrast, the absolute insulin-positive area was positively correlated with HbA1c ($r = +0.084$, $p < 0.05$; Fig. 7B).

In the T2D cohort, no correlation was identified between islet size and HbA1c and between absolute insulin-positive area and HbA1c (Figg. 7C; 7D). The percentual insulin-positive area was negatively correlated with HbA1c ($r = -0.249$, $p < 0.01$; Fig. 7D).

In the DEP cohort, no correlation was observed between the size of the islets of Langerhans area and HbA1c (Fig. 7E). The percentual insulin-positive area was negatively correlated with HbA1c ($r = -0.210$, $p < 0.01$; Fig. 7F). There was no correlation between the absolute insulin-positive area and HbA1c (Fig. 7F).

3.9 Distribution of tumour grading in the patients' groups

Non-malignant neoplasia accounted for 28% of patients with ND and 29% of patients with T2D. The lowest proportion of NM was found in the DEP group with only 9% (Fig. 8).

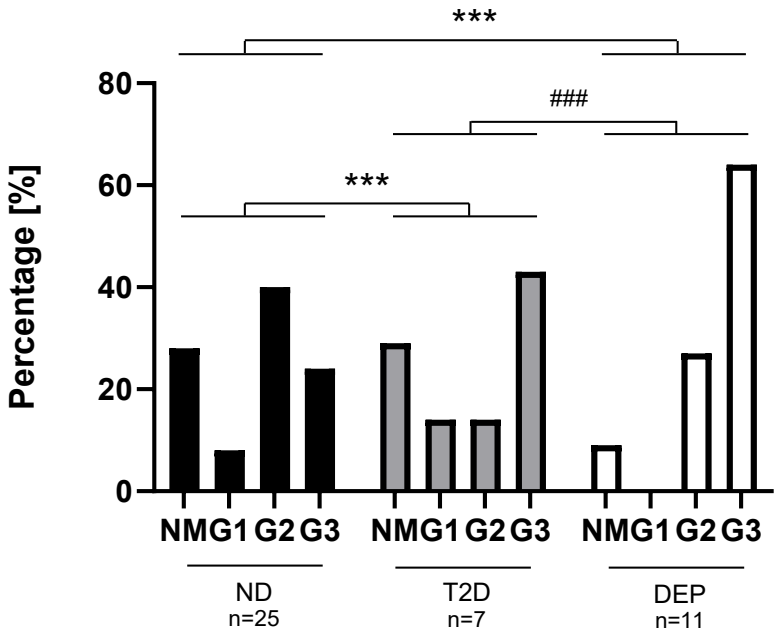

Fig. 8: Distribution of tumour grading in the patients' groups

ND: patients without diabetes mellitus; T2D: type 2 diabetes mellitus; DEP: diabetes of the exocrine pancreas; NM: non-malignant neoplasia. G1: tumour grading G1; G2: tumour grading G2; G3: tumour grading G3¸ n: number. Statistical significance (*** $p < 0.001$ compared to ND; ### $p < 0.001$ compared to T2D) is indicated. The Fisher's exact test was used to test for differences in the percentage distribution.

Ten of the patients included in the study had no malignant pancreatic neoplasia in the tissue samples analysed after pancreatectomy. In three patients, the intraoperative condition did not allow a definitive tumour grading to be determined from the histopathological report. A tumour grading could be determined for a total of 43 patients.

The percentage of G1 tumours was 8% in the ND group and 14% in the T2D group, while no G1 tumour was found in the DEP group. The distribution of G2 tumours was as follows: 40% in the ND group, which was the highest proportion in this group, 14% in the T2D and 27% in the DEP groups. For G3 tumours, the highest proportion was found in DEP group with 64%. In the T2D group, 43% of patients had G3 tumours and in the ND group only 24% (Fig. 8).

A comparison of the group-specific distribution patterns by the Fisher's exact test revealed differences. There was a difference between the distribution pattern of the DEP group and the distribution pattern of the ND group with a p-value < 0.001). There was also a difference between the distribution patterns of the DEP and T2D groups and the ND and T2D groups ($p < 0.001$) (Fig. 8).

3.10 Distribution of tumour stage in the patients' groups

Ten of the patients included in the study had no malignant pancreatic neoplasia in the tissue samples analysed after pancreatectomy. In one patient, intraoperative condition did not allow for a definitive tumour staging from the histopathological report. In total, 45 patients could be staged according to the UICC criteria (Fig. 9).

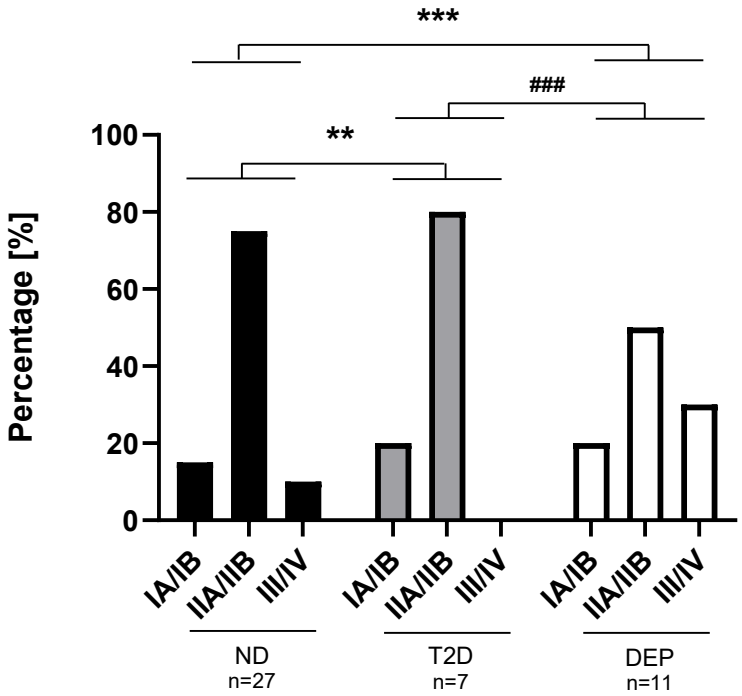

Fig. 9: Distribution of tumour stages in the patients' groups

ND: patients without diabetes mellitus; T2D: type 2 diabetes mellitus; DEP: diabetes of the exocrine pancreas; IA/IB: tumour stage IA/IB. IIA/IIB: tumour stage IIA/IIB. III/IV: tumour stage III/IV; n: number. Statistical significance (** $p < 0.01$ compared to ND; *** $p < 0.001$ compared to ND; ### $p < 0.001$ compared to T2D) is indicated. The Fisher's exact test was used to test for differences in the percentage distribution.

The percentage of stage IA to IB tumours was 15 % in the ND group, 20% in the T2D group and 20% in the DEP group. The percentage of stage IIA to IIB tumours was 75% in the ND group, 80% in the T2D group and 50% in the DEP group. Stage III/IV tumours were found in 10% of the ND group and 30% of the DEP group. None of the patients with T2D included in the study had stage III/IV tumours (Fig. 9).

When the distribution patterns of the percentage of tumour stages in the respective ND, T2D and DEP groups were analysed using the Fisher´s exact test, a difference was found between these patterns. There was a difference between the distribution pattern of the DEP group and the distribution pattern of the ND group ($p < 0.001$). There was also a difference between the distribution patterns of the DEP and T2D groups and the ND and T2D groups ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 9).

3.11 HbA1c and fasting plasma glucose levels according to tumour grading and stage

Mean HbA1c was higher in the tumour grading 3 (G3) cohort (7.0 ± 1.4%) compared to the non-malignant (NM) group (5.8 ± 0.7%), with $p < 0.05$ (Fig. 10A). Fasting plasma glucose levels were also higher in the G3 group (126.4 md/dl ± 41.9 mg/dl) compared to the NM group (94.1 md/dl ± 18.1 mg/dl), with $p < 0.05$ (Fig. 10B).

The mean fasting plasma glucose level was 94.1 ± 18.1 mg/dl in the NM group and 126.4 ± 41.9 mg/dl in the G3 group. No differences in HbA1c and fasting plasma glucose levels were observed when PDAC tumour grades 1 (G1) and 2 (G2) were compared with each other or with the NM and G3 tumour groups (Figg. 10A; 10B). No differences in HbA1c and fasting plasma glucose levels were observed between the NM group and the different tumour stages (Figg. 10C; 10D).

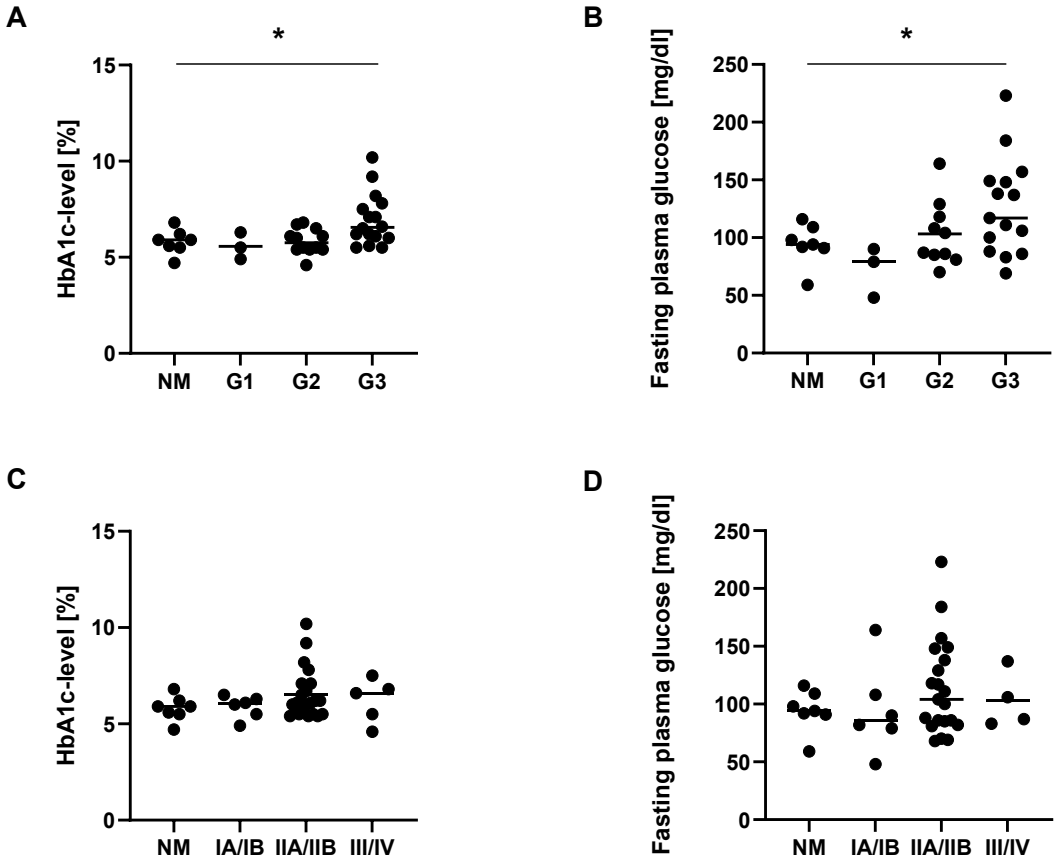

Fig. 10: HbA1c and fasting plasma glucose levels according to tumour grading and stage

NM: non-malignant neoplasia; G1: pancreatic ductal adenocarcinoma with tumour grading 1; G2: pancreatic ductal adenocarcinoma with tumour grading 2; G3: pancreatic ductal adenocarcinoma with tumour grading 3; HbA1c: Haemoglobin A1c. Mean (indicated by black horizontal line) and statistical significance (* $p < 0.05$ compared to NM) are indicated. **A:** Distribution of HbA1c across tumour gradings in the patient cohort. **B:** Distribution of fasting plasma glucose levels across tumour gradings in the patient cohort **C:** Distribution of HbA1c across tumour stages in the patient cohort **D:** Distribution of fasting plasma glucose levels across tumour stages in the patient cohort. One-way analysis of variance (ANOVA) was used for testing.

3.12 Clinical characteristics of the patients according to tumour grading

The analyses revealed no differences in HOMA-IR and HOMA-B between the NM, G1, G2, and G3 groups (Table 17). Additionally, no differences were observed in C-peptide, CRP, bilirubin, lipase, amylase and pancreatic amylase levels among the NM group and the G1, G2 and G3 groups (Table 17).

Table 18: Clinical characteristics of the patients according to tumour grading

	NM	G1	G2	G3
HOMA-IR	1.7 ± 1.3	1.8 ± 0,3	4.1 ± 4.1	4.5 ± 5.8
HOMA-B	21.9 ± 12.4	74.8 ± 33.6	40.8 ± 26.1	33.5 ± 42.6
C-peptide [nmol/l]	7.8 ± 6.5	16.8 ± 12.0	8,8 ± 7.3	7.1 ± 9.2
CRP [mg/dl]	1.8 ± 1.8	0.6 ± 0.5	4.2 ± 9.3	2.2 ± 5.9
Bilirubin [mg/dl]	0.6 ± 0.2	0.6 ± 0.3	0.8 ± 0.6	1.6 ± 3.8
Lipase [U/l]	51.0 ± 29.6	30.0 ± 5.6	83.8 ± 161.4	124.9 ± 265.9
Amylase [U/l]	66.5 ± 29.4	24.0 ± 10.6	76.4 ± 115.2	73.8 ± 77.3
Pancreatic amylase [U/l]	43.9 ± 26.3	23.0 ± 13.8	56.7 ± 116.2	49.1 ± 79.3

NM: non-malignant neoplasia; G1: pancreatic ductal adenocarcinoma with tumour grading 1; G2: pancreatic ductal adenocarcinoma with tumour grading 2; G3: pancreatic ductal adenocarcinoma with tumour grading 3; CRP: C-Reactive Protein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HOMA-B: Homeostatic Model Assessment of Beta Cell Function. Mean and standard error of the mean (SEM) are indicated. One-way analysis of variance (ANOVA) was used for testing.

3.13 Clinical characteristics according to tumour stage

Table 19: Clinical characteristics of the patients according to tumour stage

	NM	IA/IB	IIA/IIB	III/IV
HOMA-IR	2.6 ± 2.1	3.9 ± 4.9	4.5 ± 5.23	1.5 ± 0.7
HOMA-B	23.7 ± 15.9	51.7 ± 32.5	41.6 ± 40.2	18.5 ± 8.8
C-peptide [nmol/l]	7.6 ± 6.9	13.8 ± 11.5	9.2 ± 9.2	3.9 ± 1.1
CRP [mg/dl]	1.8 ± 1.8	0.5 ± 0.4	3.7 ± 8.3	0.7 ± 1.2
Bilirubin [mg/dl]	0.6 ± 0.2	0.8 ± 0.4	1.5 ± 3.2	0.4 ± 0.2
Lipase [U/l]	47.5 ± 29.6	23.8 ± 12.2	129.4 ± 251.9	47.8 ± 26.9
Amylase [U/l]	66.5 ± 29.4	33.5 ± 7.6	82.6 ± 104.6	55.0 ± 11.3
Pancreatic amylase [U/l]	43.9 ± 26.3	15.5 ± 6.6	60.7 ± 105.9	26.6 ± 8.0

IA/IB: tumour stage IA/IB. IIA/IIB: tumour stage IIA/IIB. III/IV: tumour stage III/IV; CRP: C-Reactive Protein; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HOMA-B: Homeostatic Model Assessment of Beta Cell Function. Mean and standard error of the mean (SEM) are indicated. One-way analysis of variance (ANOVA) was used for testing.

No difference was observed for HOMA-IR and HOMA-B between the NM, IA/IB, IIA/IIB and III/IV groups (Table 18).

There was also no difference in C-peptide, CRP, bilirubin, lipase, amylase and pancreatic amylase levels among the NM group and tumour stages IA/IB, IIA/IIB and III/IV (Table 18).

3.14 Tumour grading and morphological characteristics of islets in the patients' groups

No difference was observed for the area of the islets of Langerhans between patients with non-malignant neoplasia, tumour grade G1, G2 and G3 (Fig. 11A).

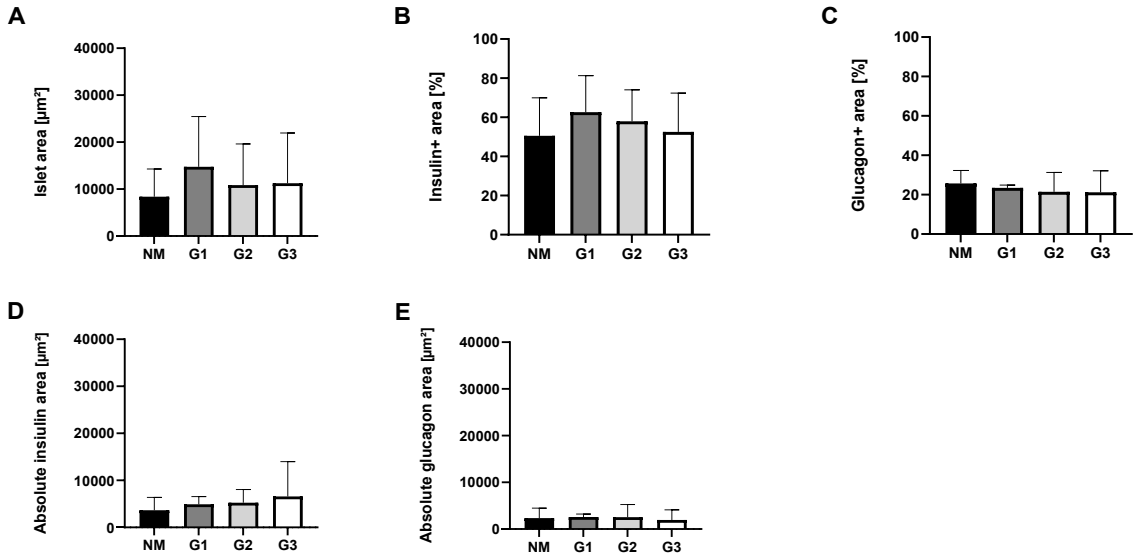

Fig. 11: Tumour grading and morphological characteristics of islets in the patients' groups

ND: patients without diabetes mellitus; T2D: type 2 diabetes mellitus; DEP: diabetes of the exocrine pancreas; NM: non-malignant neoplasia; G1: tumour grade G1; G2: tumour grade G2; G3: tumour grade G3. Mean and standard deviation are indicated. **A:** Area (μm^2) of the islets of Langerhans according to the tumour grading. **B:** Percentual insulin-positive islet area according to the tumour grading. **C:** Percentual glucagon-positive islet area according to the tumour grading. **D:** Absolute insulin-positive islet area according to the tumour grading. **E**: Absolute glucagon-positive islet area according to the tumour grading. One-way analysis of variance (ANOVA) was used for testing.

The same results were observed for insulin- and glucagon- positive areas. Both percentual insulin- and glucagon-positive areas showed no differences between non-malignant neoplasia and the different tumour grading groups (Figg. 11B; 11C). There was also no difference in absolute insulin and glucagon-positive areas between non-malignant neoplasia and the different tumour grading groups (Figg. 11C; 11D).

3.15 Tumour stage and morphological characteristics of islets in the patients' groups

The area of the islets of Langerhans did not show differences between patients with non-malignant neoplasia and patients with tumour stage IA/IB, IIA/IIB, III/A, IIIB (Fig. 12A).

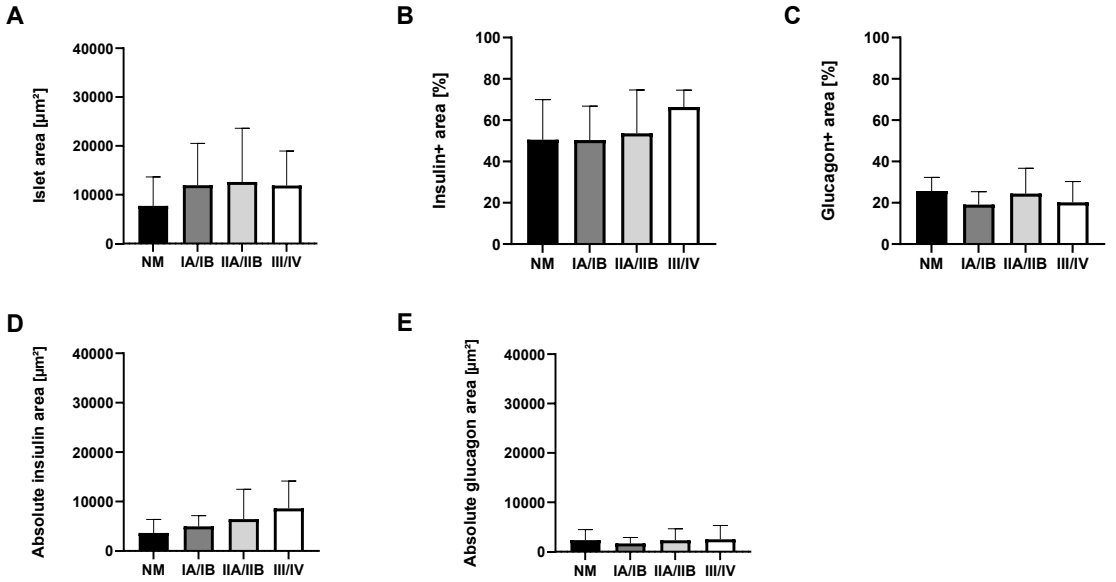

Fig. 12: Tumour stage and morphological characteristics of islets in the patients' groups

ND: patients without diabetes mellitus; T2D: type 2 diabetes mellitus; DEP: diabetes of the exocrine pancreas; NM: non-malignant neoplasia¸ IA/IB: tumour stage IA/IB. IIA/IIB: tumour stage IIA/IIB. III/IV: tumour stage III/IV. Mean and standard deviation are indicated. **A:** Area (μm^2) of the islets of Langerhans according to the tumour stage. **B:** Percentual insulin-positive islet area according to the tumour stage. **C:** Percentual glucagon-positive islet area according to the tumour stage. **D:** Absolute insulin-positive islet area according to the tumour stage. **E**: Absolute glucagon-positive islet area according to the tumour stage. One-way analysis of variance (ANOVA) was used for testing.

Similarly, no difference was observed for both percentual insulin- and glucagon-positive islet areas between non-malignant neoplasia and the different tumour stage groups (Figg. 12B; 12C). There was also no difference for the absolute insulin- and glucagon-positive areas between non-malignant neoplasia and the different tumour stage groups (Figg. 12D; 12E).

3.16 Distribution of tumour localisation and corresponding bilirubin levels in the patients´ groups

There was no difference in the distribution of tumour localisation between the ND, T2D and DEP groups. Furthermore, bilirubin levels did not differ based on the location of the pancreatic neoplasia, either when compared within the same group (ND, T2D, and DEP) or between the three groups (Table 19).

Table 20: Distribution of the tumour localisation and corresponding bilirubin levels in the patients´ groups

Tumour location and serum bilirubin levels	**ND**	**T2D**	**DEP**
Head of the pancreas [n]	15	4	9
Bilirubin [mg/dl]	2.1 ± 4.0	0.8 ± 0.2	0.4 ± 0.2
Body of the pancreas [n]	3	2	2
Bilirubin [mg/dl]	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
Tail of the pancreas [n]	5	2	4
Bilirubin [mg/dl]	0.7 ± 0.3	0.4 ± 0.1	0.6 ± 0.2

ND: patients without diabetes mellitus; T2D: type 2 diabetes mellitus; DEP: diabetes of the exocrine pancreas. Mean and standard error of the mean (SEM) are indicated. One-way analysis of variance (ANOVA) was used for testing.

4 Discussion

4.1 Introduction

Diabetes of the exocrine pancreas (DEP) is a distinct but heterogeneous group of endocrine disorders, alongside type 1 diabetes and type 2 diabetes as the two major forms of diabetes. DEP is associated with damage to the exocrine pancreas leading to a diabetic metabolic state. Acute and chronic pancreatitis are the most common causes of DEP, accounting for approximately 80% of cases (Ewald et al. 2012). However, pancreatic neoplasia, particularly PDAC, has emerged as another potential cause of DEP and is increasingly becoming in the focus of research (Hart et al. 2016). The specific pathomechanisms and tumour characteristics that influence glucose metabolism in DEP patients remain poorly understood. Therefore, this study focused on DEP associated with pancreatic neoplasia. The aim of the study was to investigate how clinical parameters and morphological characteristics of the pancreatic islets differ between patients with DEP, those with type 2 diabetes and those without diabetes, in order to improve our understanding of the interaction between glucose metabolism and tumour staging and grading in the development of DEP.

4.2 Patients´ clinical characteristics

The study was performed in a cohort of a total number of 46 patients with pancreatic neoplasia, 28 of them without diabetes mellitus, seven with type 2 diabetes mellitus known before the diagnosis of pancreatic neoplasia and eleven with type 3 diabetes mellitus were analysed. The age of the patients was homogeneous among the three groups, with an overall mean age of 68.2 ± 10.6 years at the time of diagnosis. This average age is comparable to the average age at first diagnosis of pancreatic cancer in other studies (Blackford et al. 2024; Pu et al. 2023). Based on data from the German Guideline for exocrine pancreatic carcinoma 2024, the average age of onset is 72 years for men and 76 years for women (Seufferlein et al. 2024). If the ten patients with non-malignant pancreatic tumours (e.g. with MD-IPMN) are excluded from the analysis, the average age of onset increases to an overall average of 70.0 ± 10.4 years.

Regarding the diagnosis of DEP, there are currently no universally accepted criteria. Ewald and Bretzel proposed major and minor criteria for the identification of patients with DEP as described in Table 3 (Ewald and Bretzel 2013). According to Hart et al., DEP is diagnosed when diabetes mellitus coexists with exocrine pancreatic disease and when there is a temporal relationship between the initial diagnosis of exocrine pancreatic disease and the onset of diabetes mellitus (Hart et al. 2016). In the current study, patients were classified into the DEP group based on the presence of impaired glucose metabolism according to the criteria described in 1.1.6, and its temporal relationship with the diagnosis of pancreatic cancer. In the patient cohort 39% of patients had diabetes mellitus, defined by elevated levels of fasting glucose and HbA1c. Of these, 61% were

classified as having DEP. Type 2 diabetes was already known in 39% of cases. Elevated fasting glucose was found in 48% of the cohort; 88% of patients had an elevated HbA1c above 5.7% and 56% above 6.5%.

Moreover, as expected, the study showed a significant difference in diabetes duration between patients with known type 2 diabetes and those with newly diagnosed DEP, as the classification criteria for DEP used in the study required a diagnosis of diabetes within 12 months of PDAC diagnosis. The mean duration of diabetes in type 2 diabetes patients was ten times longer than in those with DEP.

Significant differences were also observed according to the BMI distribution in the patient cohort. Indeed, in addition to genetic factors, lifestyle-related factors, particularly obesity, play a critical role in the development of type 2 diabetes. Excess body fat, especially increased visceral obesity and ectopic fat, has been shown to be a crucial pathogenic factor for insulin resistance and thus the development of type 2 diabetes (Kolb 2022; Wei et al. 2019). The BMI as an indicator of obesity has important clinical utility in predicting and monitoring type 2 diabetes and cardiovascular risk factors. Studies have reported that a BMI up to 25 kg/m^2 is associated with an increased risk for type 2 diabetes (Ha and Baek 2020; Lee et al. 2020). Bombelli et al. described an 8.4% increase in the risk of type 2 diabetes for every 1 kg/m^2 increase in BMI (Bombelli et al. 2011). In line with the literature, patients with type 2 diabetes in the current study had a higher BMI compared to ND. No difference was found between patients with DEP and ND or between patients with type 2 diabetes and DEP. In the latter case, although DEP does not appear to be associated with increased BMI, the current study showed no difference compared to type 2 diabetes, which may be due to the small number of patients included in the analysis or the fact that increased BMI is also a risk factor for developing pancreatic cancer (Seufferlein et al. 2024).

4.3 Distribution of fasting plasma glucose and HbA1c in the patient cohort

In a retrospective cohort study by Woodmansey et al., comparing glucose metabolism parameters between the different types of diabetes, patients with DEP had higher HbA1c and poorer glycaemic control than patients with type 2 diabetes (Woodmansey et al. 2017). A more recent study by Shivaprasad et al. compared patients with DEP following chronic pancreatitis with patients with type 2 diabetes and made the same observations (Shivaprasad et al. 2019). These findings were not confirmed in the present study which showed no difference in glucose metabolism parameters such as HbA1c and fasting plasma glucose between the DEP and type 2 diabetes groups. These results might be explained by the fact that the studies by Woodmansey et al. and Shivaprasad et al. had included higher numbers of participants (n= 31435 and n= 133 respectively), which may allow the detection of small differences between the groups. Moreover, the study by Woodmansey

et al. included not only patients with exocrine pancreatic neoplasia but also patients with other exocrine pancreatic diseases such as pancreatitis, haemochromatosis and cystic fibrosis, whereas the study by Shivaprasad et al. included only patients with chronic pancreatitis. However, the pathomechanisms of the different underlying diseases appear to be fundamentally different, and the resulting more inhomogeneous patient population in the study of Woodmansey et al. may contribute to the differences compared with the patient population included in the present study. Furthermore, when comparing the type 2 diabetes and DEP groups, it is important to consider that the effects of diagnosed pancreatic neoplasia on beta cell function and insulin resistance may also affect patients who were known to have type 2 diabetes at the time of enrolment. Therefore, it is possible that the observed disturbances in glucose homeostasis in patients with type 2 diabetes may be influenced by the interaction between the progressive pancreatic neoplasia and their glucose metabolism.

4.4 Insulin resistance and beta cell function in patients with PDAC

Several studies have described the presence of insulin resistance in patients with DEP (Śliwińska-Mossoń et al. 2023; Umapathy et al. 2016). In a study by Umapathy et al. analysing fasting glucose and fasting insulin in 167 patients with necrotising pancreatitis, an increased HOMA-IR, as an approximation of insulin resistance, was found compared to the control group without pancreatitis. A study by Chiari et al. analysed the different distribution of HOMA-IR in PDAC patients with normoglycaemia, impaired glucose tolerance and diabetes compared to the healthy control group without PDAC. Interestingly, HOMA-IR was also higher in PDAC patients with normoglycaemia compared to the control group without PDAC. These observations lead to the assumption that the effects of pancreatic neoplasia on insulin sensitivity may already be seen in patients with a normoglycaemic metabolic state (Chari et al. 2005b). Based on this assumption, insulin resistance was assessed in the current study by determining HOMA-IR in the subgroups of the cohort. Interestingly, HOMA-IR was found to be higher in patients with DEP compared to ND and patients with type 2 diabetes also showed higher insulin resistance than patients without diabetes. However, there was no difference between DEP and type 2 diabetes. Patients without diabetes mellitus also showed a slightly elevated HOMA-IR of 2.1 ± 2.4, supporting the observations of Chiari et al. and suggesting an impact of PDAC on insulin sensitivity even in normoglycaemic patients.

C-peptide is a marker of insulin synthesis and pancreatic beta cell function (Maddaloni et al. 2022). No difference in C-peptide levels was observed between the patient cohorts. The literature describes a normal or elevated C-peptide in patients with early-stage type 2 diabetes, which can be explained in the context of existing insulin resistance and thus compensatory increased insulin

synthesis (Vonderau and Desai 2022). In contrast, a normal to low C-peptide is described in patients with DEP, where both beta cell loss and insulin resistance are present (Vonderau and Desai 2022). According to Chiari et al. and Maddaloni et al., insulin resistance seems to play a predominant role in the early stages of DEP, whereas beta cell dysfunction mostly occurs at a later stage. For this reason, increased C-peptide can also be observed in early stages of DEP (Chari et al. 2005b; Maddaloni et al. 2022). In the current study, C-peptide levels were elevated in all three groups, supporting the assumption of insulin resistance in both, normoglycaemic and patients with diabetes and PDAC. In this context, it is possible to interpret the lack of difference in fasting plasma insulin levels between the three groups. In both, patients with and without diabetes, the average fasting plasma insulin was in the normal range (2.6 to 24.9 µU/mL). These results can be explained in the context of insulin resistance. However, since twelve patients of the cohort were on insulin treatment, the possibility of incorrect plasma insulin determinations due to external insulin administration during the 8 hours of pre-operative fasting could not be excluded.

In addition to insulin resistance, several studies have described beta cell dysfunction in patients with PDAC. The study by Chiari et al. analysed beta cell function in patients with PDAC using the Homeostatic Model Assessment (HOMA-B). Patients with PDAC and disturbed fasting glucose showed lower beta cell function than normoglycaemic patients with pancreatic cancer and without neoplasia. Beta cell function in normoglycaemic patients with pancreatic cancer was comparable to that in a control group without pancreatic neoplasia (Chari et al. 2005b). However, in the present study, there was no difference in HOMA-B between the three groups. This observation could be explained by the fact that in the DEP group the duration of pancreatic disease was shorter than in the other groups. In fact, the mean duration of pancreatic neoplasia was ten times shorter in patients with DEP compared to those with type 2 diabetes. In line with the observations of Chiari et al., the results could be explained by a predominance of insulin resistance in the early stages of DEP, as opposed to beta cell dysfunction that seems to occur only at a later stage of DEP (Chari et al. 2005b). Indeed, HOMA-B showed a negative correlation with the duration of pancreatic disease. Although there was no difference between the three groups, the mean of HOMA-B was below the normal range in all the three groups, suggesting an alteration in beta cell function due to PDAC (mean values above 50%). Several inflammatory, immune-mediated, and metabolic pathways already discussed in section 1.4 may contribute to beta cell dysfunction in the context of PDAC. However, further studies are needed to clarify the precise mechanisms involved.

4.5 Distribution of pancreatic and PDAC-related parameters in the patient cohort

The role of inflammation in tumour development has been reported in many studies, but a direct causal link has not yet been proven (Grivennikov et al. 2010; Coussens and Werb 2002, 2002; Grivennikov et al. 2010). Several hypotheses have been proposed to explain the potential mechanisms of the associations between elevated CRP concentration and risk of cancer. Possible hypotheses are based on the assumption that cancer tissue causes inflammation and thus increases serum levels of CRP, or that tumour cells produce various cytokines and chemokines that stimulate CRP production in the liver (Zhu et al. 2022). Other studies suggest that CRP is a host immune response to tumour cells or even a marker of chronic inflammation that may promote carcinogenesis (Zhu et al. 2022). According to the European Prospective Investigation into Cancer and Nutrition-Heidelberg cohort and a Danish prospective study by Allin et al., a positive association between elevated CRP and cancer was observed for all cancers, but especially for lung, breast and colorectal neoplasia (Allin et al. 2009; Srour et al. 2022). The group of Liu et al. included 52,276 patients in a study and evaluated the relationship between CRP trajectory patterns and new-onset cancers (Liu et al. 2022a). Four CRP trajectory patterns were identified: low-stable pattern, moderate-increasing pattern, increasing-decreasing pattern, and elevated-decreasing pattern. Pancreatic cancer was found to be associated with the increasing-decreasing trajectory pattern (Liu et al. 2022a). In the present study, there was no difference in CRP levels between the three groups of patients.

Furthermore, in the cohort, CRP was only measured once before the planned pancreatectomy as the study design did not include longitudinal observation of the patients. Due to the large variation in this value over time, these results may not be representative for drawing conclusions about the association of CRP with pancreatic cancer. Determining CRP levels at different intervals (e.g. at the time of diagnosis, before and after pancreatectomy) could certainly have provided important information about the relationship between inflammation and tumourigenesis but was not the primary objective of this study.

Elevated serum bilirubin levels have been frequently described in patients with PDAC (Imamura et al. 2021; Yagyu et al. 2019). In particular, when the tumour is located in the head of the pancreas, obstruction of the bile ducts leads to cholestasis and consequently elevated serum bilirubin levels (Yagyu et al. 2019). Biliary obstruction can be due to either a benign disorder or a malignant pancreatic neoplasm, but in the latter case bilirubin is known to reach higher levels (Boyd et al. 2023). Higher preoperative bilirubin levels are associated with a poorer prognosis (Imamura et al. 2021). If the cancer is located in the tail of the pancreas, there is often no obstruction of the bile ducts. Therefore, the incidence of elevated bilirubin levels is more common in PDAC of the head than of the tail (Wu et al. 2007). There was no difference in bilirubin levels between the groups

in the study. Additionally, no difference in bilirubin levels was observed according to the location of the tumour.

The current state of research is contradictory regarding the role of lipase and amylase in tumourigenesis and their value as prognostic factors. Studies have reported a highly variable behaviour of pancreatic enzymes at both low and high serum lipase levels (Asamer et al. 2018; Gültepe et al. 2016; Yagi et al. 2016). A recent study by Stotz et al. analysed lipase and amylase levels in 157 patients with PDAC before pancreatectomy (Stotz et al. 2020). Neither preoperative amylase nor lipase levels were associated with patient survival. Interestingly, the authors observed a poorer prognosis in patients with an elevated lipase/amylase ratio (Stotz et al. 2020). In the present cohort of patients, no difference in lipase and amylase levels between the non-diabetic and diabetic groups and between the different tumour stages and grades was found. There was also no difference in the lipase/amylase ratio between patients with and without diabetes, or between tumour stages and grades.

4.6 Morphological characteristics in the patients' groups

4.6.1 Size of the islets of Langerhans and insulin- and glucagon-positive areas in the patients' groups

The exact pathomechanisms leading to beta cell loss or dysfunction in patients with type 2 diabetes mellitus and DEP are still poorly understood. Studies suggest that not only beta cell function but also beta cell mass is altered (Amo-Shiinoki et al. 2021). Increased beta cell apoptosis appears to play a role in the decline of beta cell function and mass over time (Butler et al. 2003), but increased apoptosis alone does not appear to be sufficient to explain the beta cell deficit in type 2 diabetes (Kahn SE et al. 2009, Rahier et al. 2008). Recent research also highlights other mechanisms, such as islet remodelling, transdifferentiation, and dedifferentiation, which are considered important in the long-term progression of type 2 diabetes(Amo-Shiinoki et al. 2021; Tanday et al. 2024). In the study by Amo-Shiinoki et al., 26 pancreatic specimens from patients with diabetes and eleven specimens from patients without diabetes were analysed after partial pancreatectomy for resection of a pancreaticobiliary neoplasm. The results showed a reduction in beta cell mass in samples from patients with diabetes compared to those without diabetes. On the other hand, alpha cell mass was increased in patients with long-term type 2 diabetes. According to these findings, the ratio of alpha to beta cells was also increased in patients with type 2 diabetes compared to patients without diabetes (Amo-Shiinoki et al. 2021). Fujita et al. analysed the pancreatic tissue from 43 patients after pancreatectomy and observed a decrease of beta cell mass and a proliferation of alpha cells in patients with type 2 diabetes (Fujita et al. 2018). Henquin and Rahier also described a decrease in beta cell mass and an increase in the alpha to beta cell ratio in patients

with type 2 diabetes mellitus, but did not observe an increase in alpha cell mass. The higher ratio of alpha to beta cells in the islets of Langerhans was thought to be due to a decrease in beta cells rather than an increase in alpha cells (Henquin and Rahier 2011). This imbalance may contribute to alterations in the normal inhibitory influence of beta cells on alpha cells and lead to the relative hyperglucagonaemia observed in type 2 diabetes (Henquin and Rahier 2011).

On the other hand, loss of both alpha and beta cell mass has been described in patients with DEP (Aggarwal et al. 2013). Several pathways as mentioned in Chapter 1.4, such as the overexpression of adrenomedullin or the genes encoding vanin-1 and matrix metalloproteinase 9, may be involved in the impairment of endocrine cell function in DEP (Aggarwal et al. 2013; Basso et al. 2006; Huang et al. 2010).

In the current study, a reduction in absolute insulin-positive islet area in patients with type 2 diabetes and DEP compared to ND was observed. Interestingly, the percentual insulin-positive area was only reduced in patients with DEP compared to the control group. No difference in percentual insulin-positive area was observed between type 2 diabetes and ND. According to Finegood et al. and Chang-Chen et al. this result could be explained by the fact that pancreatic beta cells have the ability to largely increase their mass in response to stress conditions such as insulin resistance (Chang-Chen et al. 2008; Finegood et al. 2001). In a mouse model of insulin resistance, an initial compensatory increase in beta cell mass was observed in response to high insulin demand (Finegood et al. 2001). Early and moderate hyperglycaemia has been shown to induce beta cell replication rates to counterbalance the increasing rate of beta cell death (Finegood et al. 2001). When the compensatory mechanisms of beta cell hypertrophy are exhausted, the high beta cell replication rates cannot be maintained and beta cell mass declines (Finegood et al. 2001). In line with these findings, the type 2 diabetes cohort showed no change in percentual insulin-positive area, but absolute insulin-positive area was reduced compared to the control group.

In the present study, the glucagon-positive area was reduced in patients with DEP compared to patients with type 2 diabetes and without diabetes. Additionally, the insulin positive area was reduced in DEP compared to patients without diabetes. In type 2 diabetes, only the insulin-positive area was reduced compared to ND, while glucagon-positive area remained unchanged, resulting in a higher ratio of the glucagon-to-insulin positive area compared to DEP.

4.6.2 Correlation of diabetes duration and morphological characteristics in patients' groups

According to Hill and Hill, changes in the islets of Langerhans associated with type 2 diabetes include not only a progressive reduction in beta cell mass, but changes in alpha cell mass have also been reported (Hill and Hill 2024). Prolonged hyperglycaemia exacerbates the glucotoxic effects on islets, depleting initial compensatory mechanisms and leading to progressive islet loss

(Finegood et al. 2001). The present study revealed a reduction in the size of the area of the islets of Langerhans in long-standing type 2 diabetes. The islets of Langerhans in patients with shorter duration of diabetes were larger than those observed in patients with long-standing type 2 diabetes. In addition, smaller absolute insulin-positive areas were observed in type 2 diabetes, while percentage insulin-positive islet areas showed a positive correlation with the duration of diabetes. Although the percentual insulin-positive area was elevated in islets of long-standing type 2 diabetes, the reduced absolute insulin-positive area suggests a failure of compensatory mechanisms in long-standing diabetes, ultimately contributing to hyperglycaemia.

The role of alpha cells in type 2 diabetes is still unclear. Hyperglucagonemia has been described, but it is still unclear whether this is related to an increase in alpha cell mass (Campbell et al. 2021, Henquin et al. 2011). In the current patient cohort, there was no large variation in percentage glucagon-positive area between patients with short-term and long-term diabetes. However, the absolute glucagon-positive area was smaller in patients with long-standing diabetes. These findings contrast with previous studies reporting an increased alpha cell mass in longer standing diabetes (Henquin and Rahier 2011; Liu et al. 2020). A possible explanation for these observations is that in long-term diabetes, factors such as gluco- and lipotoxicity, amyloid deposition and cell dedifferentiation may also affect alpha cells, leading to an absolute reduction in this cell population (Hill and Hill 2024; Mizukami and Kudoh 2022). Furthermore, yet undefined tumour-related factors may also contribute to the reduction in glucagon-positive area.

A similar trend in islet size and duration of diabetes was observed in the DEP cohort. As described by Wynne, patients with DEP experience a loss of both beta and alpha cells, leading to a progressive reduction in the size of the islets of Langerhans (Wynne et al. 2019). In the current study, islet size reduction was more pronounced in DEP than in type 2 diabetes, the latter showing a stronger negative correlation between islet size and disease duration. Possible mechanisms linking exocrine pancreatic disease to altered glucose homeostasis, such as beta cell dysfunction and impaired insulin secretion induced by inflammatory or neoplastic processes have already been discussed in sections 1.1.5 and 1.4.

In contrast to type 2 diabetes, a decrease in both percentual and absolute insulin-positive islet area with increasing duration of diabetes in DEP was observed. This suggests a reduced capacity for compensatory beta cell mechanisms to counteract hyperglycaemia. In addition, as observed for the size of the islets of Langerhans, the loss of insulin-positive area was more pronounced in DEP than in type 2 diabetes. Most studies align with these findings (Sharma et al. 2018), indicating a decrease in beta cells in patients with DEP (Aggarwal et al. 2013; Katsumichi and Pour 2007; Sah et al. 2013; Wynne et al. 2019). However, a study by Tsuchiya et al., which analysed 30 autopsy cases with PDAC (with and without diabetes) and 31 cases without PDAC (with and without

diabetes), reported no differences in beta cell mass among the groups (Tsuchiya et al. 2022). Further studies are needed to clarify the impact of PDAC on beta cells.

The percentual glucagon-positive islet area in DEP remained largely unchanged with diabetes duration, whereas a negative correlation was observed between absolute glucagon-positive area and diabetes duration. Notably, Andersen et al. reported a more substantial loss of alpha cell mass in the later stages of PDAC, whereas beta cell loss predominantly occurred during the early stages of PDAC diagnosis (Andersen et al. 2017).

4.6.3 Correlation of BMI and morphological characteristics in patients' groups

The relationship between type 2 diabetes and increased BMI has already been discussed in section 4.2. However, the association between obesity and DEP is also object of current research. BMI is associated with a modest increase in the risk of PDAC, estimated to range between 10% and 50% for every five-unit increase in BMI (Aune et al. 2012; Urayama et al. 2011). Similarly, Carreras-Torres et al. observed that the risk of pancreatic cancer increased by 34% for every five-unit increase in BMI (Carreras-Torres et al. 2017).

In the type 2 diabetes cohort of the current study, there was an increase in the percentual insulin-positive islet area with rising BMI, supporting the hypothesis, that compensatory mechanisms are activated to counteract insulin resistance. However, this compensatory response appears to diminish with further increases in BMI. Specifically, the size of the islets of Langerhans and the absolute insulin-positive islet area decreased as BMI increased, possibly pointing to a failure of these compensatory mechanisms in the context of gluco- and lipotoxicity.

In the DEP cohort, both the percentual and absolute insulin-positive area correlated positively with BMI. This finding aligns with a hypothesis proposed by Carreras-Torres et al., who identified a direct relationship between increased BMI and hyperinsulinaemia in pancreatic cancer (Carreras-Torres et al. 2017). Obesity contributes to elevated insulin levels in the context of insulin resistance, which subsequently reduce insulin-like growth factor (IGF) binding proteins (Carreras-Torres et al. 2017). This reduction results in higher circulating levels of insulin-like growth factor 1 (IGF-1), a well-recognized promoter of cell proliferation (Carreras-Torres et al. 2017; Lingohr et al. 2002). IGF-1 not only drives tumour cell proliferation in the context of carcinogenesis but is also known to promote beta cell proliferation (Carreras-Torres et al. 2017; Lingohr et al. 2002). Thus, these findings may reflect an initial compensatory beta cell proliferation driven by increased BMI, mediated by elevated levels of insulin and IGF-1, in response to insulin resistance. In line with the observed increase in insulin-positive islet area, a positive correlation between islet size and BMI was observed in patients with DEP. However, further studies are

needed to clarify the relationship between BMI and morphological changes of islets and endocrine cells in patients with DEP.

4.6.4 Correlation of fasting glucose and HbA1c and morphological characteristics in patients' groups

The exact mechanisms underlying the relationship between parameters of glucose metabolism, such as fasting plasma glucose and HbA1c, and PDAC are discussed controversially in the literature. For example, a study by Pannala et al. reported no association between fasting plasma glucose levels and tumour stage (Pannala et al. 2008). In contrast, a study by Sharma et al. suggested that fasting plasma glucose levels increased with tumour volume in patients with pancreatic cancer. In addition, poorly differentiated tumours were associated with elevated fasting plasma glucose levels even at lower tumour volumes compared to well or moderately differentiated tumours. These findings suggest that higher grade tumours may induce hyperglycaemia earlier in their development (Sharma et al. 2018). To further contribute to the understanding of the relationship between parameters of glucose metabolism and the development of tumours of the exocrine pancreas, the present study analysed the association between fasting plasma glucose and HbA1c in relation to tumour grade and stage. Levels of fasting plasma glucose and HbA1c were higher in patients with poorly differentiated PDAC (G3) compared to the non-malignant pancreatic disease cohort. No differences in levels of fasting plasma glucose and HbA1c were observed between different tumour stages and grades, or between the NM group and different tumour stages. However, it is important to note that all patients included in this study had potentially resectable pancreatic neoplasia. Patients with unresectable tumours were not included in the study, which may have influenced the observed results. It remains uncertain whether more advanced tumour stages might eventually lead to more pronounced changes in glucose metabolism.

The relationship between fasting glucose levels and HbA1c in relation to morphological characteristics of the islets in the patients' groups showed a similar trend between the ND, type 2 diabetes and DEP groups. A negative correlation was observed between fasting plasma glucose and HbA1c and the percentual as well as the absolute insulin-positive islet area in ND and type 2 diabetes. In the DEP cohort, there was a positive correlation between the percentual insulin-positive area and levels of fasting plasma glucose. In contrast, there was a negative correlation between absolute insulin-positive area and fasting plasma glucose levels, while no association between absolute insulin-positive area and HbA1c was observed.

4.7 Tumour-associated changes in glucose homeostasis

4.7.1 Association of tumour grading and changes in glucose metabolism

While the association between new-onset diabetes and pancreatic cancer is well-documented in literature, specific studies directly linking new-onset diabetes with tumour grading in pancreatic cancer remain limited. However, as discussed in section 4.6.4, the findings of Sharma et al. suggest a potential association between disturbed parameters of glucose metabolism and tumour grade in PDAC (Sharma et al. 2018). Their research indicates that elevated glucose levels correlate with higher tumour grades in PDAC (Sharma et al. 2018). In line with this, Leal et al. demonstrated in their analysis of patients with low- and high-risk IPMNs that the degree of dysplasia was associated with an increased risk of diabetes mellitus in patients after resection (Leal et al. 2015). In agreement with the literature, the current study revealed differences in the distribution of tumour grading in the ND, type 2 diabetes and DEP groups. Overall, patients with DEP exhibited a higher tumour grade compared to the other groups. In contrast, the proportion of benign cystic precursors was lower in patients with DEP compared to patients without diabetes. In particular, a high proportion of G3 tumours was found in patients with DEP, with 64% of all patients with DEP having G3 tumours. In addition, higher levels of fasting plasma glucose and HbA1c in patients with G3 tumours compared to those with non-malignant neoplasia were observed. These results support the notion of more pronounced dysregulation of glucose homeostasis in patients with poorly differentiated PDAC. In contrast, there was no difference in clinical parameters such as HOMA-IR, HOMA-B, C-peptide, CRP, bilirubin, lipase and amylase between tumour grading in the different groups. Furthermore, no difference was observed between morphological characteristics of pancreatic islets and tumour grading in the patient cohort. While this study revealed a reduction in the size of the insulin-positive area in patients with diabetes *vs.* ND, no difference was observed when the distribution was analysed for tumour grading. These results suggest that while diabetes is associated with morphological changes in the islet structure, these changes do not appear to vary with tumour grading in PDAC. These observations contrast with the results of a recent study by Wang et al. The group investigated the relationship between PDAC and changes in pancreatic beta cell mass (Wang et al. 2020). Their research focused on pancreatic samples from patients with different types of pancreatic neoplasia. Specifically, the study analysed tissue from 15 patients with benign pancreatic neoplasia and 15 patients with PDAC. Neither group had a history of diabetes. The beta cell mass of the PDAC group was found to be 3.6 times lower than that of the group with benign pancreatic neoplasia (Wang et al. 2020). Contrary observations to those of Wang et al. regarding the potential impact of tumour grading on beta cells were reported

by Parajuli et al. (Parajuli et al. 2020). To investigate whether PDAC could affect pancreas endocrine functions, the group utilized the Kras mouse model of PDAC (Parajuli et al. 2020). Their study revealed that tumorigenesis leads to a progressive loss of pancreatic islet and beta cell mass but did not show an association with tumour grading. Even in a sample of a well-differentiated tumour, beta cell loss was observed (Parajuli et al. 2020). This result aligns with clinical observations of hyperglycaemia and diabetes, which often precede the diagnosis of pancreatic cancer. In conclusion, the current study found an association between more pronounced disturbance of parameters related to glucose metabolism and tumour grading, as well as an association between DEP and poorly differentiated tumours. However, no association between tumour grading and morphological changes in islets was observed. Further studies are needed to understand the pathways involved in the changes of the endocrine cell mass in patients with PDAC.

4.7.2 Association of tumour stage and changes in glucose metabolism

In pancreatitis, the most common cause of DEP, several studies have shown a correlation between the extent of pancreatic tissue damage and the reduction in insulin production, whereas in ductal adenocarcinoma no clear correlation has been described between tumour size alone or tumour stage and insulin secretion and resistance (Malecka-Panas et al. 2002; Pannala et al. 2008; Pelaez-Luna et al. 2007). According to a study by Aggarwal et al., 85% of patients had impaired fasting glucose levels as early as 36 months before PDAC diagnosis, even when the tumour was too small to be detected by routine imaging, supporting the hypothesis that diabetes associated with PDAC is not primarily due to local tumour effects such as infiltration of pancreatic tissue or increased tumour size (Aggarwal et al. 2013). In addition, glucose intolerance has been described in 60% of patients with relatively small tumours with less than 20 mm in diameter (Chari et al. 2005a). In line with these findings, the current study found no difference in the levels of fasting plasma glucose and HbA1c according to tumour staging. In addition, no difference was observed for HOMA-IR, HOMA-B, C-peptide, CRP, bilirubin, lipase and amylase levels between the different tumour staging groups. Morphological characteristics of the pancreas samples, such as the size of the islet area and the insulin- and glucagon-positive islet areas, did not differ according to tumour staging. In contrast, there was a difference in the distribution patterns of the proportions of tumour stages in the respective ND, type 2 diabetes and DEP groups. Patients with DEP had a higher tumour stage compared to the other groups, with 50% of patients with DEP having tumour stage IIA/IIB and 30% having tumour stage III/IV. Tumour stage III/IV was not present in the type 2 diabetes group and only in 10% of patients without diabetes. The relationship between diabetes and tumour volume in PDAC has been examined by Chu et al., Liu et al. and Sharma et al., revealing associations between tumour size, the prevalence of diabetes, and patient outcomes (Chu et al. 2010; Liu et al. 2022b; Sharma et al. 2018). Liu et al. described a higher presence of

diabetes with reduced median survival and higher mortality in patients with larger tumour volumes compared to smaller tumours (Liu et al. 2022b). Sharma et al. conducted a study that identified an association between parameters related with glucose metabolism and tumour volume, but this relationship was evident only for tumours larger than 1.1 cm^3. Tumours smaller than this size showed no association with fasting glucose levels. Their findings revealed that glucose-related parameters were most pronounced in larger tumours and became weaker as tumour volume decreased. Importantly, they observed that the mean fasting plasma glucose levels in cases of invasive PDAC smaller than 1 cm^3 in volume were similar to those in age- and gender-matched controls after PDAC resection. This observation suggests that invasive PDAC exceeding a specific volume threshold may be responsible for hyperglycaemia. Additionally, Sharma et al. noted that tumour grade exhibited a stronger association with glucose-related parameters than tumour volume. Notably, even though tumours larger than 1.1 cm^3 were linked to hyperglycaemia, poorly differentiated tumours of smaller size displayed even more pronounced signs of hyperglycaemia compared to well-differentiated or moderately-differentiated tumours of larger size (Sharma et al. 2018).

In summary, the existing literature consistently identifies a strong association between new-onset diabetes and PDAC. Monitoring for impaired fasting glucose or new-onset diabetes, especially in high-risk populations, may offer an opportunity for earlier detection of PDAC, potentially enabling timely intervention and improved patient outcomes. However, the relationship between metabolic changes in patients with PDAC and tumour size or stage remains less clear, with inconsistent findings across studies. This underscores the need for further research to better understand the interplay between invasive tumour behaviour and glucose homeostasis, which could refine diagnostic strategies and therapeutic approaches.

5 Limitations of the study

This study has several limitations. One major limitation is the relatively small patient cohort (n= 46), particularly the group with PDAC (n= 36), which was categorized by tumour staging and grading in the analyses. Consequently, additional statistical analyses, such as assessing the impact of other potential confounding factors, were not conducted. While the study included a control group of patients without diabetes, it did not include patients without pancreatic neoplasia. Including such a group would have been challenging, as there is no clinical indication for pancreatectomy in healthy individuals or those with diabetes alone. Another limitation is that only patients with resectable pancreatic neoplasia were included, which limits the ability to draw conclusions about more invasive and advanced tumours classified unresectable. Furthermore, patients with pancreatic neoplasia were pre-selected by the treating physicians, and some had other medical conditions or were on medications unrelated to the neoplasia, which were not accounted for in the statistical analysis.

Conversely, the study benefits from the analysis of a broad spectrum of islets of Langerhans, with a total of 1.810 islets examined. The patient cohort was homogeneous, displaying similar characteristics in terms of gender and age distribution. In contrast to many other studies, which include patients with a large variety of exocrine pancreatic diseases (e.g. pancreatitis, haemochromatosis, cystic fibrosis, pancreatic cancer), the current study was performed in a rather homogenous cohort of patients with PDAC. Furthermore, the classification of patients into the type 2 diabetes and DEP groups was based on a standardized application of widely accepted criteria, following the practice recommendations of the DDG and Hart et al. (Hart et al. 2016). Another strength of the study lies in the standardized measurement of clinical chemistry parameters, including fasting glucose, HbA1c fasting insulin, C-peptide, CRP, bilirubin, lipase, amylase, and pancreatic amylase, all conducted in a single laboratory.

Additionally, cryostat sections were prepared, and insulin- and glucagon-positive areas were stained and analysed in a blinded manner by the same individual in the same laboratory, ensuring consistency in the methodology. During patient recruitment, updates to the guidelines, such as changes to the TNM classification, were considered, and patient classification was adjusted accordingly.

6 Conclusions

The study revealed a reduction in insulin-positive area and glucagon-positive area in patients with DEP compared to those with normoglycaemia. In the type 2 diabetes cohort, although the absolute insulin-positive islet area was lower than in the control group, the percentual insulin-positive islet area showed no difference compared to the ND group. In type 2 diabetes, the absolute insulin-positive islet area showed a negative correlation with the duration of diabetes. In DEP, both percentual and absolute insulin-positive islet areas were negatively correlated with diabetes duration.

In addition, the study found an association between tumour grading and levels of both, fasting plasma glucose and HbA1c, with higher tumour grading correlating with poorer glycaemic control. Notable differences were also observed in the distribution patterns of tumour grading and staging between different patients' groups. Patients with DEP exhibited higher tumour grading and more advanced staging, suggesting a potential link between metabolic dysregulation and tumour progression. Future studies should focus on elucidating the precise pathomechanisms underlying the development of a diabetic metabolic state in patients with PDAC.

7 List of tables

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9 Bibliography

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