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Pancreatic Cancer. Glycoproteins as biomarkers. A lectin approach!

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Monografia orientada pela Professora Doutora Ana Cristina Ribeiro, Professora Auxiliar

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Resumo

O cancro pancreático é uma patologia fatal, com mau prognóstico e taxa de sobrevivência a 5 anos bastante reduzida. A sua gravidade prende-se com o seu diagnóstico tardio, que é provocado pela ausência de sintomas específicos numa fase mais antecipada e pela falta de biomarcadores específicos capazes de providenciar um diagnóstico precoce. O CA 19-9 é uma glicoproteína cujos níveis se encontram elevados nestes doentes e que corresponde, atualmente, ao biomarcador de eleição para o cancro pancreático. Todavia, este possui algumas limitações no que diz respeito à sua prestação enquanto meio de diagnóstico, já que não permite o estabelecimento de um diagnóstico precoce e não possui especificidade ou sensibilidade ótimas. Consequentemente, é urgente que sejam identificados biomarcadores capazes de detetar esta patologia numa fase mais precoce. A glicosilação (processo através do qual os glicanos são covalentemente ligados a proteínas ou lípidos, formando glicoconjugados) encontra-se alterada no cancro pancreático. As lectinas correspondem a um grupo de proteínas com uma distribuição de enorme abrangência na natureza, com capacidade de se ligarem por afinidade a recetores glicosilados. A ligação estabelecida entre lectinas e glicanos é comparável à que se observa entre anticorpos e antigénios. Assim, as lectinas representam uma ferramenta com enorme potencial na identificação de possíveis biomarcadores, sendo estes maioritariamente glicoproteínas. Esta monografia procurou recolher vários exemplos da utilização de lectinas neste contexto. Foram encontradas publicações que relacionam o aumento da expressão de glicanos e glicoproteínas com o cancro pancreático, revelando o seu potencial como biomarcadores. Outros estudos focaram-se na identificação de glicoconjugados ligados a exossomas secretados, com acumulação sérica, por doentes com cancro pancreático. Contudo, a abordagem mais promissora parece ser a que visa o estabelecimento de um painel de biomarcadores, composto por vários glicanos e glicoconjugados. Mais especificamente, foram encontradas duas descrições que identificaram, através de técnicas que envolviam o uso de lectinas, painéis de biomarcadores que demonstraram possuir uma prestação enquanto meio de diagnóstico superior à do CA 19-9. O primeiro era constituído por CA 19-9, variantes de sLeX e N-acetilactosamina sialilada do tipo 1, enquanto que o outro era constituído por CA 19-9, α-1-antiquimiotripsina, tromboespondina-1 e haptoglobina.

Palavras-chave: PDAC; Biomarcador; Glicosilação; Lectinas; CA 19-9

Abstract

Pancreatic cancer is a fatal disease with poor prognosis and low 5-year survival rate. Its severity is associated with its late diagnosis, caused by the absence of specific symptoms in its early stages and by the lack of biomarkers capable of establishing an early diagnosis. CA 19-9 is a glycoprotein which is upregulated in these patients and that serves, currently, as the main biomarker for pancreatic cancer. However, it displays some limitations in its diagnostic performance, since it cannot provide a precocious diagnosis and it does not possess optimal specificity and sensitivity. Thus, there is pressing need for biomarkers capable of detecting the disease in its early stages. Glycosylation (a process through which glycans are covalently attached to proteins or lipids, leading to the formation of glycoconjugates) is altered in pancreatic cancer. Lectins are a group of proteins with a very wide distribution in nature and that display affinity for glycosylated receptors. The attachment that is established between lectins and glycans is comparable to that which is established between antibodies and antigens. Therefore, lectins are a tool that have great potential for the identification of possible biomarkers, most of them glycoproteins. This review sought to collect several examples of the use of lectins in this context, most of which are glycoproteins. Several reports were found which connected the upregulation of glycans and glycoproteins with pancreatic cancer, revealing their potential as biomarkers. Other studies focused on the identification of glycans and glycoconjugates attached to serum accumulated exosomes secreted by pancreatic cancer patients. However, the most promising approach seemed to be the one related to the establishment of a biomarker panel made up of multiples glycans and glycoconjugates. More specifically, two reports were found that identified, using techniques involving lectins, panels of biomarkers which displayed a superior diagnostic performance to that of CA 19-9. The first was constituted by CA 19-9, sLeX variants and sialylated type 1 N-acetyllactosamine and the other was constituted by CA 19-9, α-1-antichymotrypsin, thrombospondin-1, and haptoglobin.

Keywords: PDAC; Biomarker; Glycosylation; Lectins; CA 19-9.

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Abbreviations

A1BG – Alpha-1-β glycoprotein

AACT – α-1-antichymotrypsin

AAL – *Aleuria aurantia* lectin

AAMR – Age-adjusted mortality rate

AGP – α-1-acid glycoprotein

AP – Acute pancreatitis

Asn – Asparagine

AUC – Area under the curve

BMI – Body Mass Index

BPL – *Bauhinia purpurea* lectin

C1GalT1 – Gal-transferase

CA – Celiac axis

CA 19-9 – Carbohydrate antigen 19-9

CCL2 – *Coprinopsis cinerea* lectin 2

CEA – Carcinoembryonic antigen

CF – Cystic fibrosis

CHA – Common hepatic artery

CI – Continuous infusion

CK8 – Cytokeratin 8

Con A – Concanavalin A

COSMC – Core 1 β3-Gal-T-specific molecular chaperone

CP – Chronic pancreatitis

CT – Computed tomography

CT-FNA – Computed tomography-guided fine needle aspiration

CZE – Capillary zone electrophoresis

DALYs – Disability-adjusted life years

DAMP – Damage-associated molecular pattern

DBA – *Dolichos biflorus* agglutinin

Di-sT – Di-sialyl-T

Dol-P – dolichol phosphate

ECL – *Erythrina cristagalli* lectin

ECOG – Eastern Cooperative Oncology Group

EGFR – Epidermal growth factor receptor

ELLA – Enzyme-linked lectin assay

ELISA – Enzyme-linked immunosorbent assay

ER – Endoplasmic reticulum

ERCP – Endoscopic retrograde cholangiopancreatography

ER α-Man – Endoplasmic reticulum α-mannosidase

ESMO – European Society for Medical Oncology

EUS – Endoscopic ultrasonography

EUS-FNA – Endoscopic ultrasonography-guided fine needle aspiration

FAMM – Familial atypical multiple mole melanoma syndrome

FDA – USA Food and Drug Administration

FOLFIRINOX – Folinic acid + Fluorouracil + Irinotecan + Oxaliplatin

FOLFOX – Folinic acid + Fluorouracil + Oxaliplatin

5-FU – Fluorouracil

5-FU/LV – Fluorouracil + Leucovorin (Folinic acid)

Fuc – Fucose

GAG – Glycosaminoglycan

GAL1 – Galectin-1

GAL3 – Galactin-3

GalNAc – N-acetylgalactosamine

GALNT3 – N-acetylgalactosaminyltransferase 3

Gal-T – β1,4 galactosyltransferases

GDP – Gross domestic product

GEM – Gemcitabine

GFPT1 – Glutamine–fructose-6-phosphate transaminase 1

α-Glc I – α-Glucosidase I

α-Glc II – α-Glucosidase I

GlcNAc – N-acetylglucosamine

GNAS1 – Guanine nucleotide binding protein

GnT-I–IV – GlcNAc-transferase I–IV

GSL-I – *Griffonia simplicifolia* lectin I

GSL-II – *Griffonia simplicifolia* lectin II

HA – Hepatic artery

HBOC – Hereditary breast and ovarian cancer syndrome

HBP – Hexosamine biosynthesis pathway

HDI – Human development index

HIV – Human immunodeficiency virus

HP – Hereditary pancreatitis

HPT – Haptoglobin

H. pylori – *Helicobacter pylori*

ICAM1 – Intercellular adhesion molecule 1

IL-6 – interleukin-6

IMS – Imaging mass spectrometry

IPMN – Intraductal papillary mucinous neoplasms

IVC – Inferior vena cava

JNPs – Janus nanoparticles

LacNAc – N-Acetyllactosamine

LAMP1 – Lysosome-associated membrane glycoprotein 1

LCA – *Lens culinaris* agglutinin

LDH – Lactate dehydrogenase

LEL – *Lycopersicon esculentum* lectin

Man – Mannose

α-Man I – α-mannosidase I

α-Man II – α-mannosidase II

MAL – *Maackia amurensis* lectin II

MCN – Mucinous cystic neoplasms

MDCT – Multidetector computed tomography

MRCP – Magnetic resonance cholangiopancreatography

MRI – Magnetic resonance imaging

MS – Mass spectrometry

MUC1 – Mucin-1

MUC5AC – Mucin-5AC

MUC16 – Mucin-16

Nal-IRI – Nanoliposomal irinotecan

NF-κB – Nuclear factor κB

NICE – National Institute for Health and Care Excellence

OGAs – O-GlcNAcases

OGT – O-linked GlcNAc transferase

ORP150 – Oxygen-regulated protein 150

OST – Oligosaccharyltransferase

PanIN – Pancreatic intraepithelial neoplasia

PDAC – Pancreatic ductal adenocarcinoma

PHA-L – *Phaseolus vulgaris* agglutinin-L

PhoSL – *Pholiota squarrosa* lectin

PJS – Peutz-Jeghers syndrome

PNA – Peanut agglutinin

Pro – Proline

PTL – *Pinellia ternata* lectin

PV – Portal vein

PVDF – Polyvinylidene fluoride

RCA-I – *Ricinus communis* agglutinin

RIP – Type-2 ribosome inactivating protein

RPN2 – Ribophorin 2

RSL – *Ralstonia solanacearum* lectin

SBA – Soybean agglutinin

Ser – Serine

α2,3 Sialyl-T – α2,3 sialyltransferase

α2,6 Sialyl-T – α2,6 sialyltransferase

sLeA – Sialyl Lewis A antigen

sLeX – Sialyl Lewis X antigen

SMA – Superior mesenteric artery

SMV – Superior mesenteric vein

SNA – *Sacumbus nigra* agglutinin

SRL – *Sclerotia rolfsii* lectin

ST3GalII – Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 2

ST3GalIV – Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 4

STL – *Solanum tuberosum* lectin

sT – Sialyl T antigen

sTn – Sialyl Tn antigen

TED – Thyroid eye disease

TF antigen – Thomsen-Friedenreich antigen

TGF-β – Transforming growth factor- β

THBS1 – Thrombospondin-1

Thr – Threonine

TLR – Toll-like receptor

TNF – Tumour necrosis factor

UDA – *Urtica dioica* lectin

UEA – *Ulex europaeus* agglutinin

UICC – Union for International Cancer Control

ULN – Upper limit of normal

US – Ultrasonography

VVL – *Vicia villosa* lectin

WGA – Wheat germ agglutinin

XELOX – Capecitabine + Oxaliplatin

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1 Introduction

The most common form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC) (1,2). It remains a deadly disease with dismal prognosis and low 5-year survival rate, representing the seventh leading cause of cancer-related death worldwide (3–6). Its deadliness is related with the fact that it is commonly diagnosed at an advanced stage. This can be attributed to either the presence of vague symptoms or the complete absence of symptoms when the tumour is still localized and to the fact that there are, currently, no biomarkers able to provide an early diagnosis (4).

Currently, the most widely used biomarker for pancreatic cancer (henceforth referred to as PDAC) diagnosis and monitoring is CA 19-9, since it is upregulated in PDAC patients (7–9). It is also the only FDA (USA Food and Drug Administration) approved biomarker for that effect (10,11). However, it displays some limitations in what diagnostic performance is concerned, mainly because it does not possess optimal specificity and sensitivity (8,9,12,13). In fact, CA 19-9 is upregulated in other benign conditions such as bile duct obstruction and pancreatitis (12–14). Moreover, it has also been observed that patients with negative Lewis genotype (which represent 10% of Caucasians) are unable to express CA 19-9 (10,11,15).

Furthermore, resection represents the only potential cure for this malignancy, but only 20% of patients are candidates for surgery (16). Additionally, PDAC incidence and death rates have been increasing worldwide and are projected keep increasing in the next few decades, along with the number of disability-adjusted life years (17–19).

Given all the reasons that were stated above, there is an urgent need for highly specific circulating biomarkers capable of providing an early detection, more appropriate risk stratification and personalized clinical management (8,9). One strategy for the discovery of novel biomarkers lies on the identification of specific glycosylation changes in PDAC.

Glycans occupy a vital role in cellular physiology and are often covalently attached to proteins or lipids, forming glycoconjugates named glycoproteins and glycolipids, respectively (20,21). They are obtained by a process termed glycosylation. In homeostatic conditions, glycosylation is a highly controlled and efficient process (22–24). However, this process has been shown to display several aberrations in malignant conditions, particularly in PDAC (8,25–29). Therefore, targeting specific glycosylation alterations characteristic of PDAC could present itself as a viable option in order to identify novel biomarkers.

Lectins are a ubiquitous and diverse group of non-immune proteins that are able to recognize and reversibly bind to specific glycan and glycoconjugates without altering their

structure (30–35). The interaction between a lectin and a glycan or glycoconjugate is comparable to that of an antigen and an antibody or that of an enzyme and a substrate (34,36). Moreover, lectins present a good performance in *in vitro* assays using small amounts of sample, susceptibility to recombinant production, and the necessary precision and throughput for biomarker studies (37–39). Therefore, they are ideal candidates for the identification of specific glycosylation aberrations in the pursuit of novel glycan-based biomarkers for PDAC.

2 Objectives

This review has the goal of discussing potential novel biomarkers for pancreatic cancer, namely those that correspond to glycans and glycoproteins and that have been identified by lectin-based techniques. To that end, this review aims to provide a comprehensive characterization of pancreatic ductal adenocarcinoma (the most common form of pancreatic cancer), a general description of glycosylation events, a description of the glycosylation aberrations observed in pancreatic cancer, a discussion of the glycoproteins and glycan which have been identified by the employment of lectins, a characterization of lectins as whole, as well as their classification, and finally a description of lectin-based techniques which have been used for the discovery of novel biomarkers.

3 Methods

Research was performed from January to June 2022. The bibliography was in its entirety researched on digital databases, namely Google Scholar, Researchgate, and PubMed. The information present in this paper is the result of the revision of the selected bibliography, which was duly cited. The original sources of the consulted reviews were also cited.

Searched terms included: “pancreatic cancer”, “clinical presentation of pancreatic cancer”, “diagnosis of pancreatic cancer”, “epidemiology of pancreatic cancer”, “aetiology of pancreatic cancer”, “risk factors for pancreatic cancer”, “pathophysiology of pancreatic cancer”, “pancreatic cancer therapeutic guidelines”, “glycosylation”, “glycosylation changes in pancreatic cancer”, “biomarkers for pancreatic cancer”, “biomarkers for pancreatic cancer and lectins”, “lectins”, “molecular structure of lectins”.

4 Pancreatic Cancer

PDAC remains a highly fatal malignancy with a low 5-year survival rate, representing the seventh leading cause of cancer-related deaths worldwide (3–6). Its deadliness lies on the fact that it is often discovered at an advanced stage (due to the presence of vague symptoms or lack thereof when the tumour is still localised and due to the current lack of biomarkers able to detect the carcinoma with precocity) (4). Moreover, it can quickly invade surrounding tissues and organs (3–6). Patients are often divided into one of four categories based on the extent of the disease: resectable, borderline resectable, locally advanced, and metastatic (4,16). Risk factors include age, gender, non-0 blood group, genetic factors, diabetes, smoking, high alcohol consumption, obesity, microbiota dysregulation, and pancreatitis (1,3–5).

After physical examination and clinical history analysis, should there be a suspicion of PDAC, diagnosis may be obtained through the use of several imaging techniques which include computed tomography (CT), multidetector computed tomography (MDCT), ultrasonography (US), endoscopic ultrasonography (EUS), magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), and endoscopic retrograde cholangiopancreatography (ERCP) (40–45).

Although surgery represents the only potential cure for PDAC, only 20% of patients afflicted by the disease are considered to have a resectable tumour (16). Thus, treatment, depending on resectability status, patient performance status and disease progression, revolves around administration of gemcitabine or FOLFIRINOX (16,43,45,46).

4.1 Epidemiology

As it has implications for preventive measures and clinical care, it is necessary to evaluate and comprehend recent epidemiological trends (47). Both the incidence and death rates of PDAC have been rising on a global level, some much so that it has been projected to become the third leading cause of death from cancer in the European Union (17,18). The global age-standardised incidence rate corresponded to 5.0 cases (95% UI 4.9–5.1) per 100,000 person-years in 1990, having increased to 5.7 cases (5.6–5.8) per 100 000 person-years in 2017 (18). From 1990 to 2020, the total number of cases increased 2-3 times, from 195,000 to 495,773. Likewise, the number of deaths has had an increase of 125% in the same period (18,48). This trend will most likely remain relevant in years to come due to the aging of the population (18).

Additionally, incidence and mortality rates seem to vary according to the HDI (human development index) level of a certain region. In recent decades, geographic regions with higher

HDI levels and higher GDPs (gross domestic product) have been found to possess higher age-standardized incidence and mortality rates (6,18,48,49). In the USA, a total of 60,430 new cases and 48,220 deaths have been reported, according to Cancer Statistics 2021 (6,50). In the European Union, the number of new cases was 59,000 in 1990 and 109,000 in 2019. In the same period, the number of deaths went from 60,000 to 109,000 and the disability-adjusted life years (DALYs) went from 1.3 million to 2.0 million. By 2039, is it projected that incident cases, the number of deaths and the DALYs will reach 147,000, 155,000, and 2.7 million, respectively (19). Conversely, the lowest rates were observed in South Asia. In 1990, this region's age-standardized death rate was 1.6 per 100,000 person-years in 1990 and 2.9 in 2017 (18). Despite such differences in incidence and mortality, variations in 5-year survival rates between high-income and low and middle-income regions are less pronounced, not exceeding 10% (6,18,51).

On a national level, Portugal has also seen a significant increase in the number of deaths caused by PDAC, going from 701 in 1991 to 1415 in 2015, which translates into a mean annual increase of about 3%. However, it should be noted that the increase rate was three times higher in the latter half (1.56%) than in the previous one (0.37%). Interestingly, it was also possible to observe some regional differences in mortality inside the country. Namely, the Azores Islands and Alentejo displayed the highest AAMRs (age-adjusted mortality rates) (52).

4.2 Aetiology and Risk Factors

4.2.1 Non-Modifiable Risk Factors

4.2.1.1 Age

Age is one of the most significant risk factors for PDAC. Most commonly, it affects older adults. Roughly 9 out of 10 newly diagnosed individuals are older than 55 years and most of them are fall into the 70-80 age group (3,6,18,51). In men, incidence and mortality peak between the ages of 65 and 69, while in women these metrics peak between the ages of 75 and 79 (18).

4.2.1.2 Gender

Regarding gender, the worldwide incidence of PDAC is superior in males than it is in females (5,6,49). The difference appears to accentuate in countries with higher HDI levels (3,51). It is unclear why such differences are observed. It could be that females are less prone to develop PDAC in general, or that they have lower exposure to certain environmental risk factors, namely smoking (1,18). Evidence shows that smoking habits are about 5 times more prevalent in males (25.0%) than in females (5.4%) (18,53).

4.2.1.3 Blood Group

There is growing evidence that certain blood group antigens affect the risk of developing PDAC (3,6,54). More specifically, individuals belonging to the non-0 blood group seem to have a higher risk of developing PDAC (6,55,56). Interestingly, a meta-analysis found that the risk was markedly increased with all non-0 blood types in populations not endemic for CagA-positive strains of *H. pylori (Helicobacter pylori)*. However, it was also observed that there was only increased risk for group A in endemic populations (57).

4.2.1.4 Genetic Factors

Over the years, several studies have shown that genetics play an important role in PDAC and family history significantly increases its risk of occurrence (3,58,59). In 10%-15 % of all PDAC cases, a hereditary cancer predisposition syndrome can be implicated (60). Such syndromes include Hereditary Breast and Ovarian Cancer Syndrome (HBOC), Lynch syndrome, Peutz-Jeghers Syndrome (PJS), Familial Atypical Multiple Mole Melanoma Syndrome (FAMMM), Hereditary Pancreatitis (HP), and Cystic Fibrosis (CF) (1,4,60).

HBOC is usually caused by mutations in the genes BRCA1 or BRCA2. The relative risk of PDAC is higher for the latter (3.5%) than for the previous (2.8%), when compared to the general population (1.3%) (4). Lynch Syndrome is caused by one of the four mismatch repair genes: MLH1, MSH2, MSH6 and PSM2 (58). The elevated risk of PDAC seems to have a higher degree of association with the first two and, to a lesser extent, the third (58,61). PJS is caused by a mutation in the tumour suppressor STK11, resulting in a 35% increase in the risk of PDAC (4,62). FAMMM is characterized by a mutation in CDKN2A and is associated with a 17% increase in the risk of PDAC (4). 4 out of 5 cases of HP are associated with a mutation to the PRSS1 gene (1). This, in turn, leads to an increase in trypsinogen autoactivation in the pancreatic tissue, culminating in acute and chronic pancreatitis (58). Cancer onset is triggered by chronic pancreatic inflammation. Some studies identified a significantly increased risk for malignancy for patients with HP when compared to the general population (63). CF is associated with the CFTR gene mutation, and its pathogenic mechanism is similar to the one seen in HP (1).

4.2.1.5 Diabetes

While PDAC is itself considered to be a risk factor for diabetes, the latter has long been linked to the development of this type of cancer (4). Individuals who have had type 1 diabetes for more than 10 years have a 5-10 times higher risk of developing PDAC (3). For individuals who have had type 2 diabetes for more than 5 years, there is a relative risk of 1.5 of developing

PDAC whereas that same risk increases to 5.4 in individuals diagnosed with that same condition for less than 1 year (64). Consequently, new-onset diabetes seems to be an important risk factor for PDAC (4,64).

4.2.2 Modifiable Risk Factors

4.2.2.1 Smoking

Smoking is the most significant modifiable risk factor for PDAC (3). The pathogenic mechanism seems to involve mutations to the KRAS and p53 genes and chronic inflammation. Both these factors lead to the production of cytokines and growth factors which, in turn, lead to cell transformation (1). It is estimated that 11%-32% of PDAC deaths can be attributed to smoking (18). Even though the proportion of age-standardised deaths in both sexes caused by smoking has decreased over the last decades, it remains higher than those attributable to high fasting plasma glucose and high BMI (body mass index) (18). Smokers are nearly twice as likely to develop PDAC than non-smokers (4,5). Moreover, the risk increases with the duration of the tobacco use over the years, as well as with the number of cigarettes smoked per day (5). Smoking may also influence the effect of other risk factors, such as alcohol (65).

4.2.2.2 Alcohol Consumption

The results for the association of alcohol consumption and PDAC are inconsistent. Many studies suggest an association between high alcohol consumption (more than three drinks per day) and the development of PDAC, while the same association is not established for low and moderate alcohol consumption (5). A meta-analysis reporting data from 4,211,129 individuals found a relative risk of 1.15 (95% CI) for a high consumption of alcohol (more than 24 g/day). That same analysis found no significant risk for a moderate (12-23.9 g/day) or low consumption (less than 12 g/day) (66). However, a recent hospital based-control study showed no significant association between alcohol drinking and an increased PDAC risk, even at high levels of alcohol consumption (67).

Moreover, there is also data that may suggest a possible causal relationship between the association of alcohol intake and tobacco smoking and PDAC. *Rahman et al.* (2015) found that current smokers who practiced a high-level intake of alcohol had an age-adjusted odds ratio of 4.04 (95% CI: 1.58 – 10.37), whereas that risk was not observed in non-smokers who practiced that kind of alcohol consumption (65). Despite such findings, the association of alcohol consumption and smoking is quite close, so it may be difficult to implicate alcohol as an independent risk factor for this type of cancer (5).

4.2.2.3 Obesity

Several studies have linked obesity to an increase in PDAC incidence and mortality (3,4,18,68). This risk appears to be higher in obese individuals of both genders, when compared to individuals with a healthy BMI (5). The pathogenic mechanism seems to be related with adiposopathy, which leads to the production of pro-inflammatory cytokines and hormonal imbalances – namely higher levels of leptin and adiponectin (69). Fatty infiltration of the pancreas has also been implicated in the development of PanINs (pancreatic intraepithelial neoplasias), which are precursors for the development of PDAC (4,70).

4.2.2.4 Microbiota

Some studies have demonstrated a close relation between oral, gastrointestinal and pancreatic human microbiota alterations and the occurrence and prognosis of PDAC (3,6,71,72). The involvement of said microorganisms could be manifested either through microbiota-induced innate immune suppression and adaptive immunosuppression, the promotion of malignant transformation by microbial metabolites such as lipoteichoic acid and secondary bile acids, dysregulation of the human microbial systems, and association of microbial toxins and virulence (3,51).

Regarding the oral microbiota, the levels of *Streptococcus mitis* and *Neisseria elongate* were found to be lower in patients with PDAC (73). Conversely, it has also been found that the mean relative proportions of Firmicutes and related taxa were higher in patients with PDAC while the mean relative proportions of Proteobacteria and related taxa were higher in control groups (6,74). Moreover, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were associated with a higher risk of PDAC, while the phylum Fusobacteria and its genus *Leptotrichia* were associated with a decreased risk of said cancer (6,75).

On the other hand, there have been studies which confirmed the association between the gastrointestinal microbiota and known risk factors of PDAC, such as obesity and diabetes. This may suggest a relationship between it and PDAC (6,76). The main microorganisms that contribute to the development and progression of PDAC are the Hepatitis B Virus, the Hepatitis C Virus, and *H. pylori* (3).

Although the pancreas has long been considered a sterile organ (due to the presence of proteases and due to the alkaline nature of the pancreatic juices), it has been found that patients with PDAC display a 1000-fold increase of bacteria in the pancreatic tissue when compared to tissue from healthy individuals (77). Further, a comparative study showed an important increase

in the number of *Bifidobacteria*, *H. pylori*, *Clostridium*, and Gammaproteobacteria in the pancreas of patients with PDAC (3). The latter could be related to the drug resistance of gemcitabine (78).

4.2.2.5 Pancreatitis

Pancreatitis – CP (chronic pancreatitis) in particular – has long been thought to be a risk factor for PDAC, due to its association with chronic inflammation of this tissue (79). It has been found that the risk of PDAC increases significantly after an initial diagnosis of pancreatitis, regardless of its type (acute or chronic), decreasing as time goes by (80). Moreover, an increase in the number of AP (acute pancreatitis) episodes has also been associated with an increased risk of PDAC (80). However, the probability of a relapse is 20%-30% higher after a single episode and 10% of relapsing cases do progress into CP (6,81). Further, patients who progress to CP have a 9-fold increase in the risk of developing PDAC when compared to those who don't (82). It may lead to debilitating pain, episodes of AP, endocrine and exocrine deficiency, as well as precancerous lesions, such as PanINs, IPMNs (intraductal papillary mucinous neoplasms), and MCNs (mucinous cystic neoplasms) (3–5,79,83).

4.3 Clinical Presentation

Often, this type of cancer causes either few or no manifestations in its early stages (4,5). This, coupled with its biological aggressiveness, leads to delayed diagnosis in more than 80% cases (43). Additionally, it is common for those who do develop symptoms to have unspecific complaints, such as epigastric or back pain, nausea, bloating, abdominal fullness or change in stool consistency, which often end up being attributed to alternative benign causes (4,84). The most common clinical features at the time of diagnosis are abdominal pain (40%-60%), abnormal liver function tests (about 50%), new-onset diabetes (13%-20%), dyspepsia (about 20%), nausea or emesis (about 16%), back pain (about 12%) and weight loss (about 10%) (85).

Clinical presentation may also be dependent upon the primary location of the tumour within the pancreas (Figure 1) (4,16,43). Tumours that develop in the head or neck of the pancreas (which represent 60%-70% of tumours) have a higher likelihood of causing biliary obstruction, leading to jaundice, which in turn causes steatorrhea (due to exocrine pancreatic insufficiency), hepatic dysfunction (which could lead to hepatic failure, if untreated) nausea, vomiting, dark urine and itching (16,43,68,86,87). Tumours developed in the pancreatic body tend to invade local vascular structures, namely the celiac, hepatic, and superior mesenteric arteries, as well as the portal vein, which leads to abdominal pain that exudes into the sides of

the back (4,86). Finally, tumours that develop in the tail frequently exhibit an unhampered growth due to a reduced number of neighbouring anatomical structures, which leads to a more advanced tumour at the time of diagnosis (4,43).

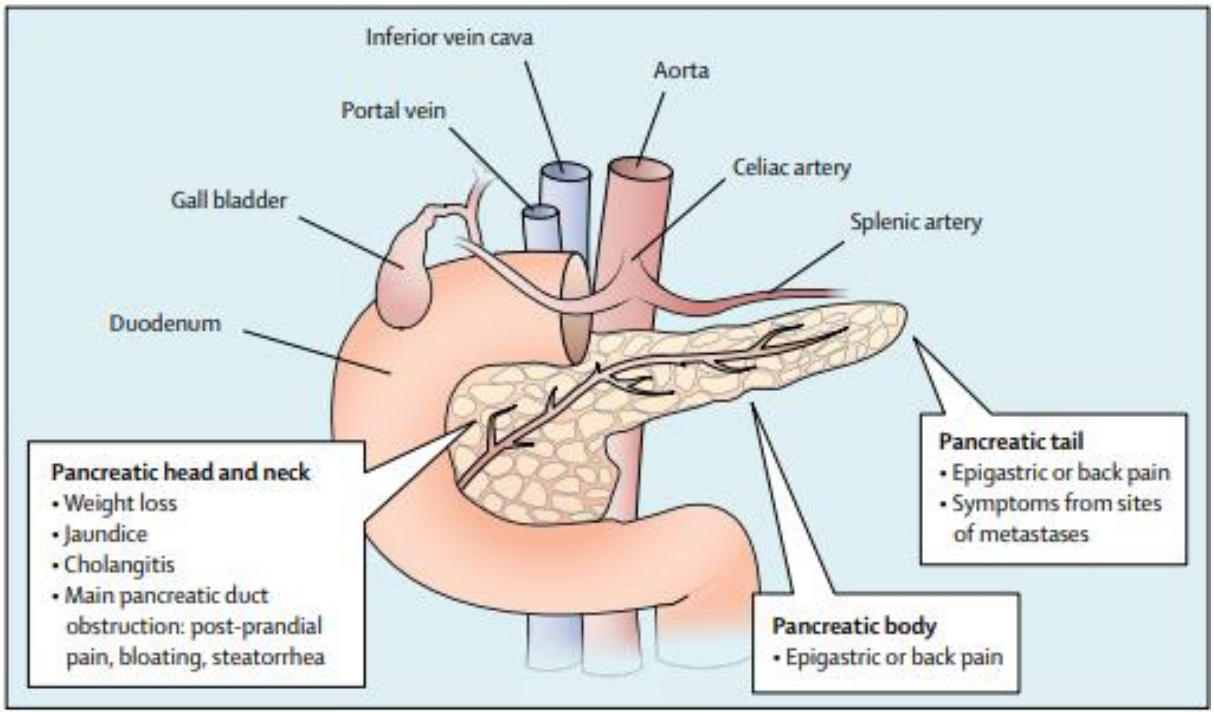

Figure 1 Common signs and symptoms by site of pancreatic tumour.
Signs and symptoms vary according to the primary location of the tumour within the pancreas. Anatomically, this organ can be divided into four sections: head, neck, body, and tail.
[From *Mizrahi et al.* (2020), with permission from Elsevier (4)].

4.4 Pathophysiology

Emerging evidence shows a strong association between inflammation and PDAC (88). Chronic inflammation of the pancreatic tissue is associated with the occurrence of precancerous lesions, which lead to the development of PDAC (79). Taking their morphology, biology, and clinical behaviour into account, we can identify three distinct precursor lesions: PanINs, IPMNs, and MCNs (86).

PDAC is most frequently originated by PanIN lesions (89). They occur in the pancreatic ducts at a small calibre, measuring less than 5 mm in diameter (86). In turn, three distinct PanIN lesions can be identified: PanIN-1 (PanIN-1A/PanIN-1B), PanIN-2, and PanIN-3. Different genetic alterations can be found in each type of lesion. KRAS (the best known oncogene with the highest mutation rate) mutations can be found in PanIN-1 and PanIN-2 (86,90). The latter also displays inactivation of tumour suppressor genes CDKN2A and p16INK4A, which are generally involved in arresting cell cycle at G1 phase (86). PanIN-3 lesions may exhibit mutations in SMAD4 (tumour suppressor gene inactivated in more than 50% of PDAC cases), TP53 (tumour suppressor that induces growth arrest or apoptosis), BRCA2 (gene involved in

cell cycle, cytokinesis, centrosome duplication, and cell death), and DPC4 (tumour suppressor gene inactivated in 55-80% of PDAC cases) are also observed (86,91–93).

IPMNs are cystic neoplasms which arise in the head of the pancreas (86). Mutations observed in these lesions include KRAS mutations in codon 12 or 13 and guanine nucleotide binding protein (GNAS1) mutations (94). In later stages, however, mutations in the TP53, CDKN2A, and SMAD4 can also be found.

MCNs, on the other hand, consist of thick mucin and haemorrhagic material without communication with the native pancreatic ductal system. Genetic alterations include KRAS mutation at codon 12, in earlier stages, and SMAD4, in later stages (86).

Besides mutations to suppressor genes and to genes involved in cell cycle regulation, cytokines have been shown to play a role in carcinogenesis (88). In fact, chronic inflammation states may lead to the activation of certain signalling pathways which in turn lead to the upregulation of proinflammatory cytokines such as interleukin-6 (IL-6) (95). Moreover, damage-associated molecular patterns (DAMPs) appear to activate Toll-like receptors (TLRs) during pancreas inflammation (96). This activation leads to the release of NF-κB (Nuclear Factor κB) which supports the inflammatory microenvironment (97).

4.5 Diagnosis and Staging

Upon suspicion of PDAC, based on medical anamnesis and/or physical examination, diagnosis is obtained primarily through several imaging modalities which include CT and MDCT, US and EUS, MRI and MRCP, and ERCP (42,43,45,98–101). Staging, on the other hand, can be obtained based on these imaging studies, as well as pathological studies (43).

CT (preferably MDCT), if a dual-phase protocol performed with an arterial and a portal venous phase is employed, is the preferred tool for dedicated pancreatic imaging, since it has high accuracy to detect and to stage pancreatic malignancies (43,45,46,98,102,103). However, it does have some drawbacks. Not only it relies on radiologists' experience for a correct interpretation of the results, it also has reduced sensitivity to detect lesions smaller than 2 cm (98,103). Besides, ionizing radiation exposition is a factor that should be considered (98). There are no significant differences between CT and MRI/MRCP for pancreatic staging in what sensitivity and specificity are concerned. Thus, the latter's use is not widespread due to its elevated cost and reduced availability (43,46). For that reason, it is mainly used as an alternative to CT in very specific cases (43,45,46).

Although its sensitivity for the detection of pancreatic tumours is low (ranging between 50% and 70%), an abdominal US is often the first test performed in patients who present

abdominal pain and/or jaundice, since it is able to detect bile duct or pancreatic duct dilation and the presence of pancreatic mass (43). Due to its inherit risks related to sedation and invasiveness, EUS is still not recommended as a routine staging tool for PDAC (46). However, it may occupy a complementary role in staging and diagnosis, as it facilitates the examination of primary tumours, relationship with neighbouring structures and tissue sample acquirement for pathological diagnosis (43,45,46). Besides, there have been several reports over the last decade which account for its superiority over MDCT (including the detection of small tumours) (3,98,104,105). Additionally, it provides high-resolution images and can characterize solid and cystic lesions with accuracy (44). It has also been known to be useful in screening asymptomatic precancerous branch duct IPMNs, large PanINs and PDAC (98). EUS-guided fine-needle aspiration (EUS-FNA) displayed diagnostic accuracy for malignancy in more than 85%-90% of cases (98). In fact, it is preferred over CT-guided fine needle aspiration (CT-FNA) in patients with resectable disease because of better diagnostic yield, safety, and potential lower risk of peritoneal seeding with EUS-FNA when compared with the percutaneous approach (46). It also proved to be useful in cytological diagnosis of IPMN and MCN in up to 70% of the cases (98).

ERCP is typically reserved for cases of bile duct obstruction due to its adverse effects and low profitability in achieving histological diagnosis (16,43).

Surveillance and screening should be offered to individuals with HP and PRSS1 mutation, with BRCA1, BRCA2, PALB2 or CDKN2A (p16) mutations, and one or more first-degree relatives with PDAC, with PJS, with two or more first-degree relatives with PDAC, across two or more generations or with Lynch syndrome and any first-degree relatives with PDAC (40,45).

Biomarkers will be discussed in greater detail ahead (see section 5.3). However, as of yet, there is no biomarker with high enough specificity and sensitivity to carry out routine clinical diagnosis and screening of PDAC (41,98). Carbohydrate antigen (CA) 19-9 is the most widely used blood-based biomarker in clinical practice and also the only one approved by the FDA for progression and therapeutic response monitoring (98).

4.6 Treatment

Although disease may be may be staged according to the TNM classification after an established PDAC diagnosis, it is more useful from a management perspective to classify them into resectable, borderline resectable, locally advanced unresectable and metastatic (III,A) (16,43,46). The R classification was established in 1987 by the UICC (Union for International Cancer Control) with the purpose of characterizing treatment efficacy according to the residual tumour after surgical resection. According to this classification, an R0 surgical resection implies

complete remission (106). It represents the only potential curative treatment for PDAC and should be attempted whenever possible (16,43,46). Nevertheless, less than 20% of patients have a resectable tumour (16,46). Resectability criteria are described in

Table 1.

Table 1 Criteria for resectability in PDAC

Criteria	Arterial	Venous
Resectable	No tumour contact with the CA, SMA or CHA.	No tumour contact with the SMV or PV or ≤ 180° contact without vein contour irregularity.
Borderline Resectable	Pancreatic head/ uncinate process: • Solid tumour contact with the CHA without extension to the CA or HA bifurcation allowing for safe and complete resection and reconstruction; • Solid tumour contact with the SMA of ≤ 180°; • Presence of variant arterial anatomy (e.g. accessory right HA and presence and degree of tumour contact should be noted as it may affect surgical planning). Pancreatic body/tail: • Solid tumour contact with the CA of ≤ 180°; • Solid tumour contact with the CA of > 180° without involvement of the aorta and with intact and uninvolved gastroduodenal artery.	• Solid tumour contact with the SMV or PV of >180°, contact of ≤ 180° with contour irregularity of the vein or thrombosis of the vein but with suitable vessels proximal and distal to the site of involvement allowing for safe and complete resection and vein reconstruction; • Solid tumour contact with the IVC.
Unresectable	• Distant metastasis. Pancreatic head/ uncinate process: • Solid tumour contact with SMA > 180°; • Solid tumour contact with the CA > 180°; • Solid tumour contact with the first jejunal SMA branch. Body and tail • Solid tumour contact with the SMA and CA; • Solid tumour contact with the CA and aorta.	Pancreatic head/ uncinate process: • Unreconstructible SMV/PV due to tumour involvement or occlusion (can be due to tumour or bland thrombus); • Contact with most proximal draining jejunal branch into the SMV. Body and tail • Unreconstructible SMV/PV due to tumour involvement or occlusion (can be due to tumour or bland thrombus).

Abbreviations: CA – celiac axis; SMA – superior mesenteric artery; CHA – common hepatic artery; SMV – superior mesenteric vein; PV – portal vein; HA – hepatic artery; IVC – inferior vena cava.
[Adapted from *Ducreux et al.* (2015) (16)].

4.6.1 Treatment of Resectable and Borderline Resectable Disease

Neoadjuvant treatment is administered in order to increase overall survival by increasing the rate of R0 resection and early treatment of micrometastatic disease (43). It may also avoid unnecessary surgical resection in patients with aggressive tumours which develop early progression (43). However, it is vital that bile duct drainage is normalized prior to treatment (43). Endoscopic placement of a metal stent is the procedure of choice to mitigate obstructive jaundice (III,B) (43).

Neoadjuvant therapy should only be considered in patients with resectable disease who are part of a clinical trial (43,45). The same is recommend for patients with borderline resectable disease by the National Institute for Health and Care Excellence (NICE) Guidelines for Pancreatic cancer in adults: diagnosis and management (45). However, it is recognised as a therapeutic option in these patients by Hidalgo *et al*. (2017) (II,B) (43). The adopted chemotherapy should be associated with higher response rate in patients with metastatic disease (gemcitabine/nab paclitaxel, FOLFIRINOX) (43,107,108). There is no current evidence to recommend one over the other. Therefore, decision should be based on patients' characteristics and centre of care experience (43). It is generally administered for 3-4 months with reassessment performed by a multidisciplinary team afterwards (II,B) (43). Patients with responding tumours could proceed to surgical resection (II,B) (43,109,110). Radiotherapy alone is not recommended and should be combined with either fluoropyrimidines or gemcitabine (II,B) (43). Patients with documented metastatic progression cease to be candidates for surgery and should be managed accordingly (II,B) (43).

As aforementioned, R0 surgical resection represents the only curative option and should be attempted whenever possible. Before surgery is performed, a complete assessment of operative risk should be carried out (due to its inherit high morbimortality) (43). It is also vital that an adequate nutritional status is attained. In order to achieve this, the administration of nutritional supplements or even parental nutrition to malnourished patients 1 to 2 weeks before surgery should be considered (43).

If the tumour is resectable, extension to adjacent organs does not represent a contraindication for surgery (43). However, the type or surgery is dependent upon the size and location of the tumour (16). Tumours localized in the head of the pancreas are usually treated with pancreatoduodenectomy (Whipple procedure), as well as with standard lymphadenectomy (16,43,45). Tumours of the body or tail should be treated with distal pancreatectomy (16,43). Some studies seem to show a reduction of morbidity when less invasive techniques (such as

laparoscopy) are employed, but data relating to these techniques is still scarce (16,111). For that reason, open surgery remains the standard of care (II,C) (16).

Adjuvant treatment should be initiated some time after surgery, so patients may recover. Therefore, it should be initiated within the next 12 weeks after surgery, in patients without active infection, serious postsurgical complications or signs or symptoms of recurrent disease (43,45). Additionally, prior to its administration, a series of tests should be carried out, which include a complete blood count, a renal function test, the determination of albumin, LDH (lactate dehydrogenase) and CA 19-9 levels, as well as a CT scan to ascertain lack of disease progression (16,43,112–114). The administration of adjuvant treatment in patients who received neoadjuvant treatment is still up for debate (16,43,115,116). Those cases should be analysed by a multidisciplinary team, but generally these patients should receive adjuvant treatment for a total of 6 months (III,B) (43).

Both the ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of pancreatic cancer and the Consensus guidelines for diagnosis, treatment and follow-up of patients with pancreatic cancer in Spain recommend either gemcitabine or 5-FU (fluorouracil) with folinic acid for a total of 6 months (I,A) (16,43,117–120). However, the ESPAC-4 clinical trial has also shown that the overall survival of patients that were treated with a combination of gemcitabine and capecitabine was superior to those treated with gemcitabine alone (121). The NICE guidelines for pancreatic cancer recommend gemcitabine plus capecitabine to people who have had sufficient time to recover after resection and gemcitabine alone for those who are incapable of tolerate combination chemotherapy (45).

Regarding chemoradiation, no trial has yet demonstrated any benefits, not even in R1 patients. Thus, it should not be given to patients after surgery outside of clinical trials (I,E) (16).

4.6.2 Treatment of Locally Advanced Disease

When treating patients with locally advanced PDAC, the main goal is to improve survival (43). Suitability for treatment relies on factors such as ECOG (Eastern Cooperative Oncology Group) performance status, age, good nutritional status, the presence of adequate bilirubin levels, lack of comorbidities, and absence of signs and symptoms of local tumour growth (43). These may include pain and bowel or bile duct obstruction. The latter should be corrected prior to treatment initiation (43).

Treatment of this stage of PDAC remains disputed, due to the lack of data from well controlled randomized trials (16,43). The NICE guidelines for pancreatic cancer recommend the use of combination chemotherapy for patients who are well enough to tolerate it and

gemcitabine for those who are not. They also recommend using capecitabine as a radiosensitiser when chemoradiotherapy is used (45). The ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of pancreatic cancer recognizes 6 months of gemcitabine as the standard of care in these cases (I,A). They also do not recommend any chemoradiation treatment besides classical combination of capecitabine and radiotherapy (IV,C) (16). The Consensus guidelines for diagnosis, treatment and follow-up of patients with pancreatic cancer in Spain states that, for patients who are candidates to chemotherapy treatment without any limitations, current trends are to use either a combination of gemcitabine and nab paclitaxel or FOLFIRINOX for a period of 3-4 months, followed by assessment of tumour response (43). If there is a partial response that allows surgical resection, the latter could be counted as a treatment option. If there is partial response and stable disease but surgical resection remains impossible, chemotherapy treatment, consolidation with chemotherapy and radiotherapy are plausible options (43). Patients who are candidates to chemotherapy with limitations have a dismal prognosis and should be treated with either gemcitabine alone, gemcitabine combined with nab paclitaxel or radiation therapy alone (43).

4.6.3 Treatment of Metastatic Disease

Like in other disease stages, treatment for advanced stage disease will vary according to factors including ECOG performance status, nutritional status, age and comorbidities (43). However, it is generally treated with systemic chemotherapy (16,43). It is important to add that patients should be included in clinical trials whenever possible (43). Treatment of metastatic disease is summarized in Figure 2.

Regarding first line treatment, the Consensus guidelines for diagnosis, treatment and follow-up of patients with pancreatic cancer in Spain and the ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of pancreatic cancer recommend that patients who are able to receive chemotherapy without limitations should be treated with either combination of gemcitabine and paclitaxel or FOLFIRINOX (I,A) (16,43,107,108). However, FOLFIRINOX should not be given to patients over 75 and it is associated with higher toxicity and thromboembolic complications (43). Candidates to chemotherapy with limitations should be administered gemcitabine combination therapy until disease progression or unacceptable toxicity are manifested (43). Those who are not candidates for chemotherapy should receive palliative treatment (16,43). In turn, the NICE guidelines for pancreatic cancer recommend FOLFIRINOX for patients with ECOG performance status 0-1 and gemcitabine combination

therapy only for those who cannot tolerate the former. They also recommend gemcitabine in monotherapy for those unable to tolerate combination therapy (45).

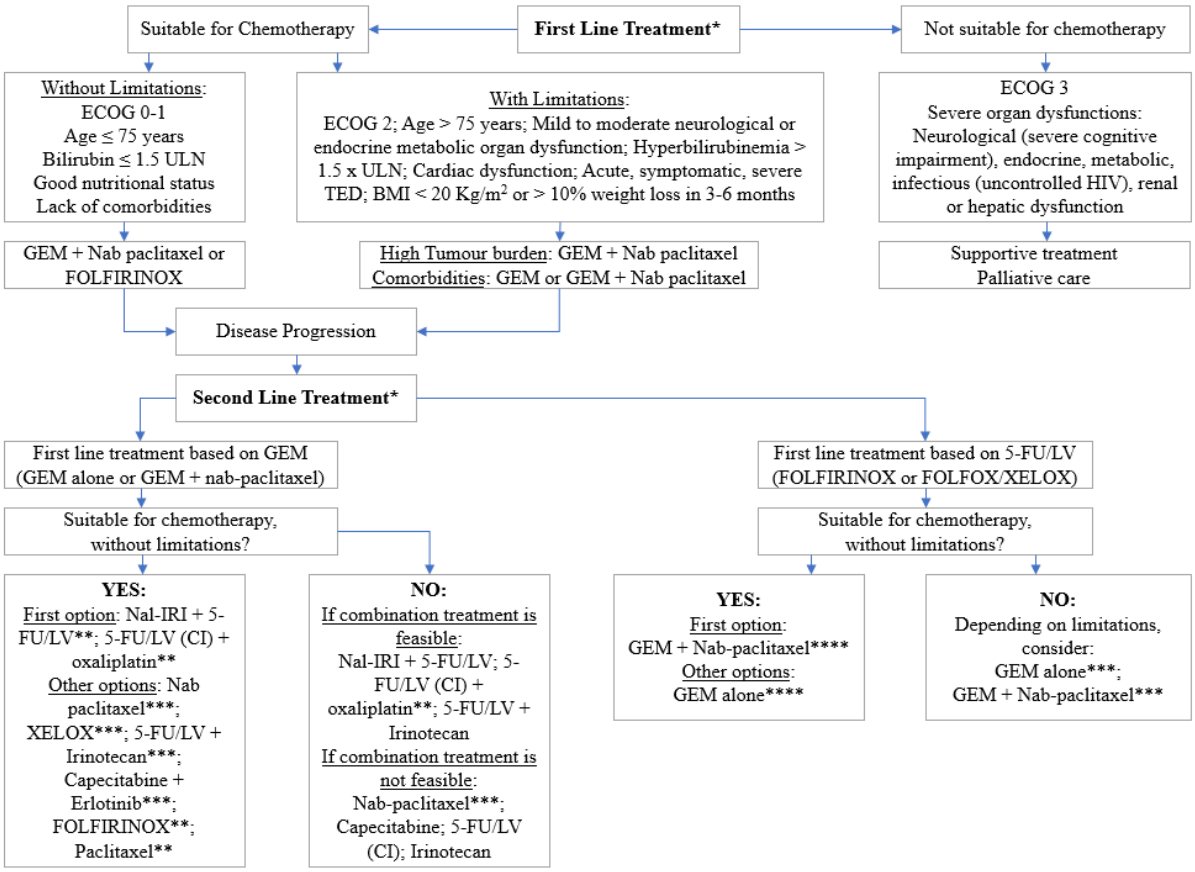

Figure 2 Algorithm for the treatment of metastatic PDAC.
*Inclusion in a clinical trial should always be considered **Based on phase III studies ***Based on phase II studies ****Based on retrospective studies. Abbreviations: ECOG – Eastern Cooperative Oncology Group; ULN – Upper limit of normal; TED – Thyroid Eye Disease; BMI – Body Mass Index; HIV – Human Immunodeficiency Virus; GEM – Gemcitabine; Nal-IRI – Nanoliposomal Irinotecan; 5-FU/LV – Fluorouracil + Leucovorin (Folinic Acid); CI – Continuous Infusion; FOLFIRINOX – Folinic Acid + Fluorouracil + Irinotecan + Oxaliplatin; XELOX - Capecitabine + Oxaliplatin; FOLFOX - Folinic acid + Fluorouracil + Oxaliplatin.
[Adapted from *Hidalgo et al.* (2017) (43)].

Second line treatment is dependent upon patients' general status and first line treatment (43,45). Typically, second line treatment for patients treated with a first line gemcitabine-based regimen corresponds to an oxaliplatin-based regimen and vice versa (43,45). In the CONKO-003 trial, patients treated with a first line gemcitabine regimen and second line treatment with FOLFOX displayed improved survival when compared to second line treatment with 5-FU (122). There is less data to support clinical choices regarding patients treated with first line 5-FU/LV. For that reason, gemcitabine or gemcitabine combination therapy are usually recommended (123). Treatment monitoring should be performed every 8-12 weeks through a CT scan of the thoracic, abdomen and pelvic regions (III,B) (43). CA 19-9 should also be quantified every 4-8 weeks (III, B). Should such measurements indicate the tumour progression, a confirmation should be obtained radiologically (III,B) (16,43,107,108,124).

5 Glicosylation

5.1 Glycosylation Events and Cellular Glycome

Glycans occupy a vital role in the cellular physiology (20,21). They are most often found covalently bound to proteins or lipids, forming glycoconjugates termed glycoproteins or glycolipids, respectively. The entire repertoire of glycoconjugates produced by a given cell is referred to as the glycome (21,125). These molecules can either be secreted or cover the cell surface, forming a multifunctional layer called glycocalyx (14,20,126). They are involved in a wide array of cellular activities, which include signal transduction, protein folding and quality control, antigenic masking in viral infection, recognition events, and metabolic roles (127–132). Additionally, the composition of glycoproteins varies according to organism, tissue, and cell type (12,126).

Glycoconjugates are obtained by a process called protein glycosylation. In the case of protein glycosylation, a glycan is attached to a specific amino acid residue from the side chains of a protein (129). It takes place mostly in the secretory pathway of the cell, which involves the endoplasmic reticulum (ER) and the Golgi apparatus (22). Unlike proteins, glycan sequences are not encoded genetically (14). Their biosynthesis is instead regulated by a wide array of factors such as the availability of nucleotide sugars that act as donor substrates (e.g., UDP-galactose, UDP-N-acetylglucosamine, GDP-fucose, and CMP-N-acetylneuraminic acid), acceptor substrates, cofactors, and the necessary glycosyltransferases and glycosidases to catalyse such reactions (14). The main types of protein glycosylation in humans are N-glycosylation and O-glycosylation (12,127,133,134). The main components of human N and O-glycans are fucose (Fuc), galactose (Gal), mannose (Man), GlcNAc (N-acetylglucosamine), GalNAc (N-acetylgalactosamine), and N-acetylneuraminic acid (12).

Regarding N-glycosylation, it occurs when there is a GlcNAc is attached via β-glucosylamine linkage to the nitrogen atom of an Asn (asparagine) side chain, following the consensus motif Asn-X-Ser/Thr (asparagine-X-serine/ threonine), where X corresponds to any amino acid except Pro (proline) (133,135). N-glycans are branched and share a common pentasaccharide core, made up of two GlcNAc residues and three mannose residues, which facilitates its recognition when methods like mass spectrometry are employed (20,133,135).

The process of N-glycosylation is illustrated in Figure 3. It relies upon the formation of a lipid precursor constituted by a branched carbohydrate structure made up of mannose and GlcNAc units attached to a dolichol phosphate (Dol-P) on the cytoplasmic side of the ER. Afterwards, this precursor is flipped into the ER lumen, where mannose and glucose units are

added. The carbohydrate chain is then added to a protein at the designated site. It suffers additional processing inside the ER, mainly involving the removal of glucose and mannose residues. Subsequently, the structure is moved into the cis-Golgi compartment, where it is processed by a series of mannosidases. Complex and hybrid N-glycans are finally formed in the medial and trans-Golgi compartments. This is possible due to the action of glycosidases and transferases, which leads to the addition of GlcNAc, galactose, sialic acid or fucose (133,136).

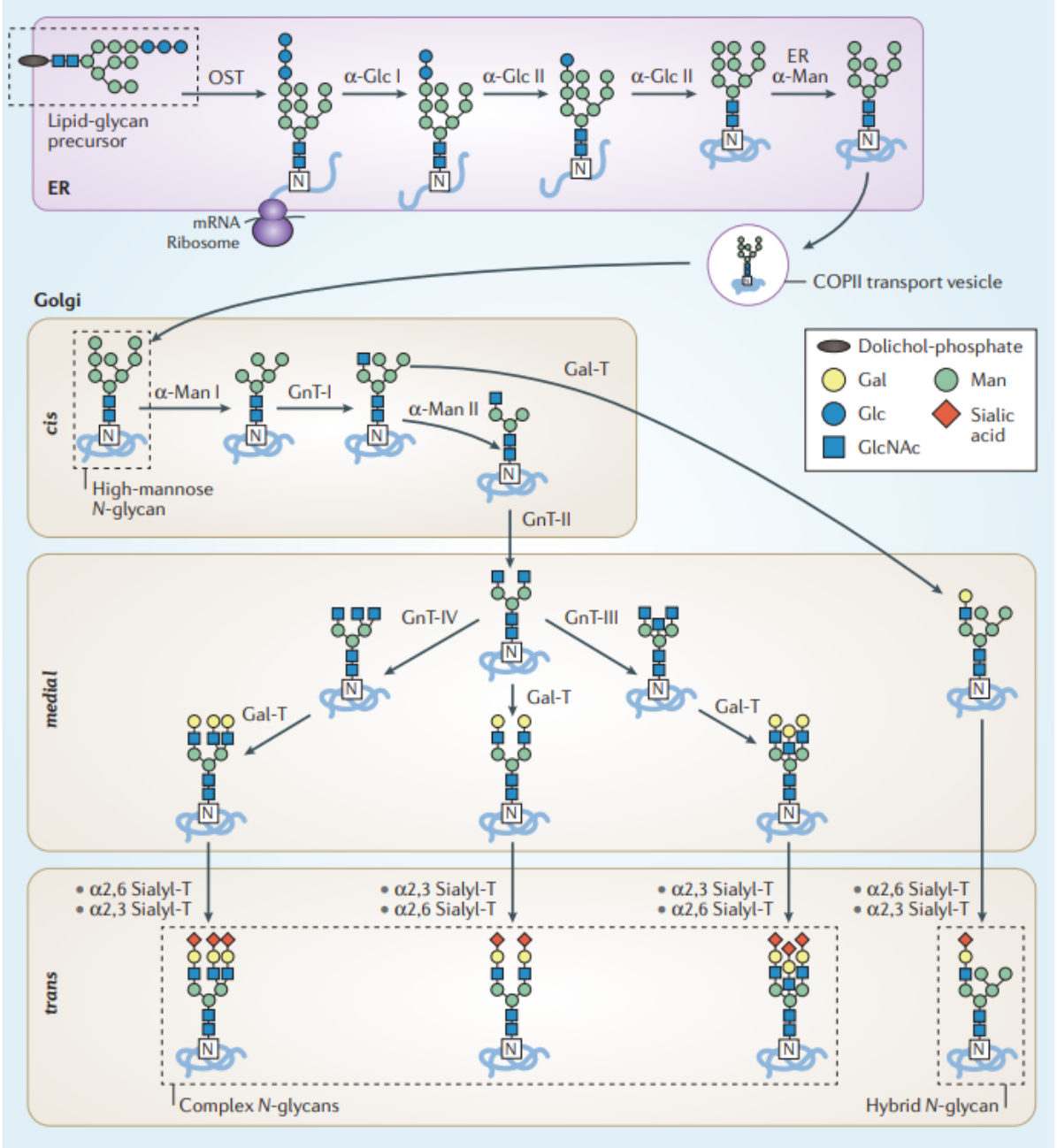

Figure 3 N-glycan biosynthesis in the secretory pathway.
Synthesis initiates in the ER with the transfer of a precursor - $Glc_3Man_9(GlcNAc)_2$ – bound to a Dol-P to an Asn residue by the multisubunit OST (oligosaccharyltransferase). There is a subsequent sequential removal of the glucose residues by two α-glucosidases (α-Glc I and α-Glc II) and of the initial mannose residue by the ER α-mannosidase (ER α-Man). After a quality-control checkpoint, the glycoprotein is moved into the Golgi apparatus for further trimming by α-mannosidase I and II (α-Man I–II) and further glycan modifications by glycosidases and transferases — GlcNAc-transferase I–IV (GnT-I–IV), β1,4 galactosyltransferases (Gal-T), α2,3 sialyltransferase (α2,3, Sialyl-T) and α2,6 sialyltransferase (α2,6 Sialyl-T).
[From *Riley et al.* (2019), with permission from Springer Nature (133)].

On the other hand, O-glycosylation occurs when there is an attachment of GlcNAc or GalNAc to functional hydroxyl groups from Ser or Thr residues (133). O-glycans can either be linear or branched and, contrary to what is observed in N-glycans, have no consensus motif and no pentasaccharide core (20,135).

Figure 4 illustrates GalNAc O-glycan biosynthesis and capping. GalNAc-linked O-glycans, also known as mucin-type O-glycans, are quite abundant on many extracellular and secreted glycoproteins (133,137,138). Regarding its biosynthesis, a family of 20 GalNAc-transferases is responsible for the first step of mucin-type O-glycosylation (which takes place in the Golgi apparatus), forming the GalNAcα1-Ser/Thr linkage in O-glycoproteins (22,137). They are then elongated or branched into various core structures which are themselves further extended and capped by several terminal structures (22). There are 4 major core structures: cores 1-4 (139). Core 1 (also referred to as T antigen) is obtained by the addition of a galactose residue to the GalNAc-O-Ser/Thr structure (also referred to as Tn antigen), which is catalysed by a Gal-transferase (C1GalT1) (22,139,140). Core 2 is obtained when the core 1 structure is branched with GlcNAc residue, which is catalysed by the C2GnT enzyme family (22,141). Although it is less common, core 3 may be formed by the addition of a GlcNAc residue to the Tn antigen by a GlcNAc-tranferase from the C3GnT family and core 4 may be formed by the addition of a second GlcNAc residue to the resulting structure (22,139,142). These core structures frequently receive further extension and are terminally capped with sialic acid or fucose residues. Moreover, in tumour contexts, the T and Tn antigens are frequently capped with sialic acid, forming sialyl-Tn (sTn), sialyl-T (sT) and di-sialyl-T (Di-sT) antigens (22). The terminal saccharides capping the O-glycans will determine which specific roles certain glycan epitopes will occupy. Such roles may include cell-cell interaction, protein recognition and molecular partnership, transmembrane receptor activation, pathogen binding and immune cell modulation (22).

On the other hand, O-GlcNAc glycans are usually not extended and they can be found in the nucleus, mitochondria, and the cytoplasm (133). Contrary to what was seen in mucin-type O-glycans, O-GlcNAc glycans' biosynthesis typically does not take place in the Golgi apparatus and is regulated by OGTs (O-linked GlcNAc transferases) and OGAs (O-GlcNAcases) (133,143). OGT uses UDP-GlcNAc as a substrate, which is provided by the hexosamine biosynthesis pathway (HBP) (8,14,133). It is also important to add that the nutritional and metabolic status of a given cell will influence O-GlcNAcylation (14).

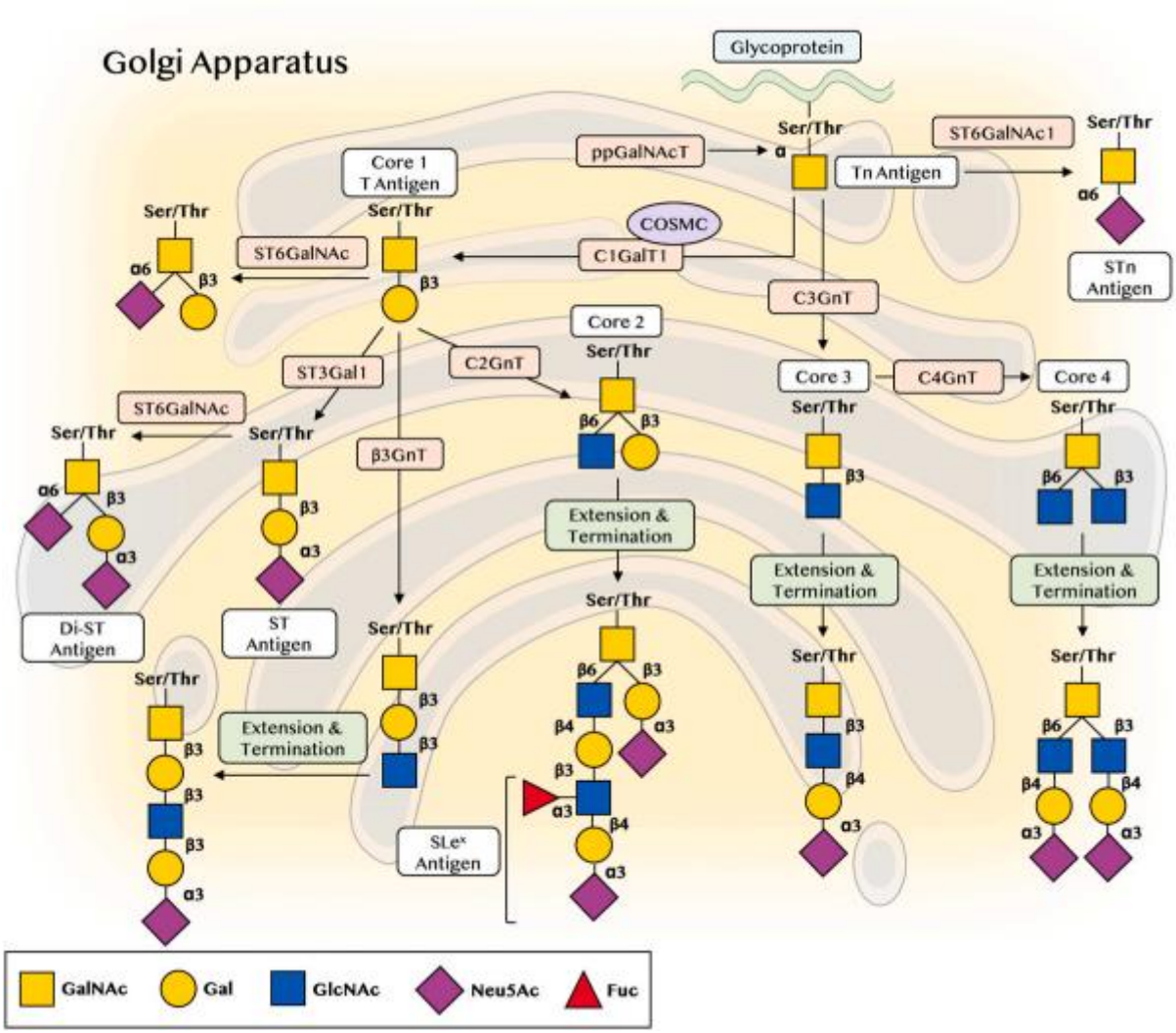

Figure 4 GalNAc O-glycan biosynthesis and capping.
[From *Magalhães et al.* (2021), with permission from Elsevier (22)].

5.2 Glycome and Glycosylation Aberrations in Pancreatic Cancer

In homeostatic conditions, glycan biosynthesis is highly controlled and efficient and leads to the normal glycosylation of proteins (22–24). Nevertheless, major cellular glycosylation changes are associated with certain pathologic conditions (22). In fact, protein glycosylation has been shown to be altered in several malignancies and some studies have shown that glycans play a role in regulating tumour proliferation, invasion, metastasis, and angiogenesis (8,25–29). As common as glycosylation aberrations in cancer are, evidence suggests that somatic mutations in genes that control cellular glycosylation are actually very rare and few glycosyltranferase mutations have been reported (128).

In case of a healthy pancreas, glycoproteins play a key role in the protection and lubrication of the pancreatic ducts (8,144). When PDAC develops, however, protein glycosylation becomes deregulated, and the ensuing altered expression of specific glycans is

associated with disease progression and poor prognosis (8). For example, *Chugh et al.* (2016) found that the altered glycosylation of the EGFR (epidermal growth factor receptor) and ErbB2 led to increased aggressiveness (145). Specifically, the cancer-induced downregulation of GALNT3 (N-acetylgalactosaminyltransferase 3) led to the association of short truncated Tn and T antigens to the EFGR and ErbB2 receptors, resulting in increased malignant features in PDAC cell lines, namely increased cell motility and adhesion to the tumour epithelium (145,146).

Specific glycome aberrations in PDAC include increased sialylated glycans, namely sialyl Lewis antigens A and X (sLeA and sLeX), increased truncated O-glycans (Tn and sTn antigens), increased fucosylated and aberrantly branched N-glycans, upregulation of specific proteoglycans and galectins, and increased O-GlcNAcylation (Figure 5 and Table 2) (8,14,147–149). Interestingly, a study which performed lectin microarray was used to perform differential glycomic profiling of crude extracts derived from non-tumour and tumour regions found that two noticeable features of PDAC, namely increases in sialylated glycans and bisecting N-acetylglucosamine and decreases in ABO blood group antigens (147).

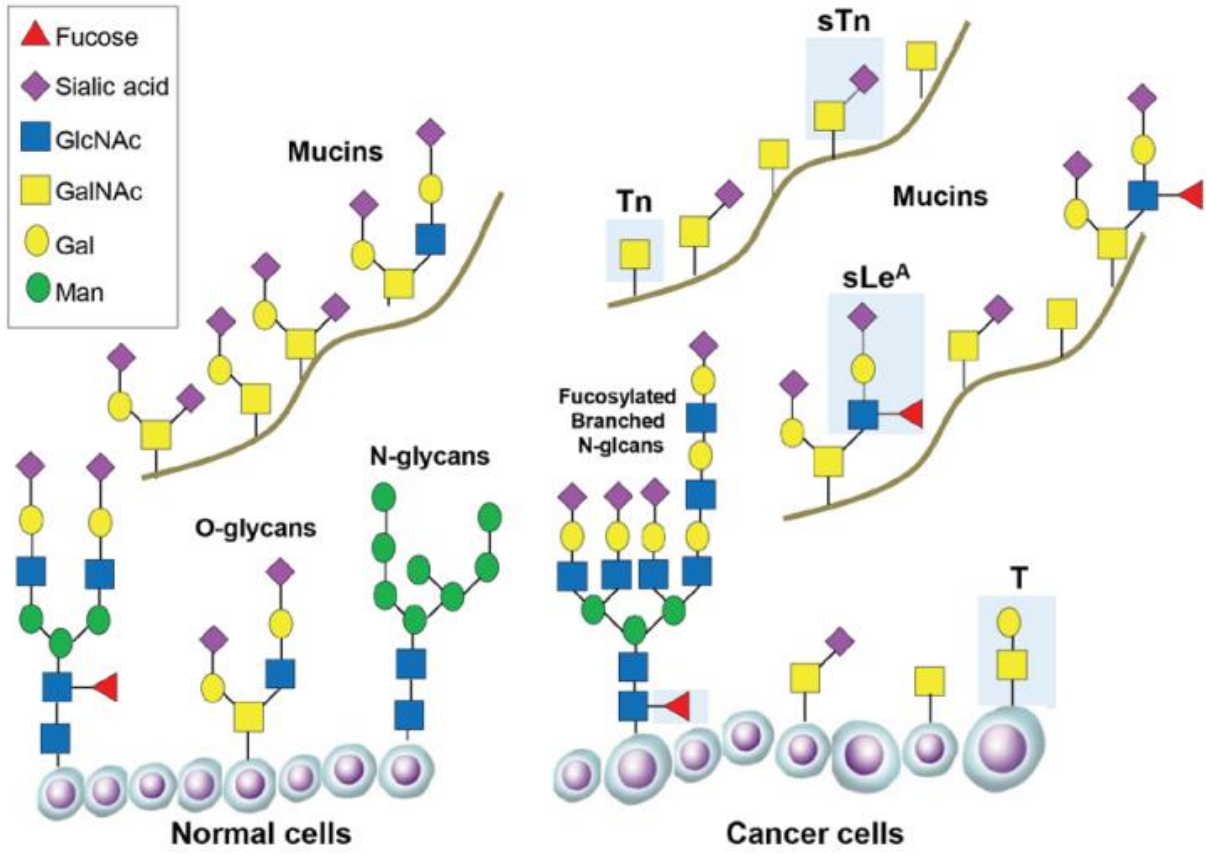

Figure 5 Changes in glycosylation during cancer progression.
Representative O-glycans and N-glycans are shown attached to the surface of normal and cancer cells. O-glycans are also shown attached to mucins. Additionally, relevant tumour-associated glycans are shown in the blue boxes, including truncated O-glycans (T, Tn and sTn) and fucosylated branched N-glycans (sLeA and SLeX).
[Adapted from *Munkley, 2019* (8)].

Sialyl Lewis antigens (namely sLeA and sLeX) play important roles in PDAC. In fact, sLeA, also referred to as CA 19-9, is the current biomarker of choice for PDAC diagnosis and monitoring, given its overexpression in most patients afflicted by the condition (10,11). The exact mechanism responsible for the elevation of the CA 19-9 levels is not understood in its entirety, but it seems to be related to the dysregulation of sialyltransferases, such as Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 2 and 4 (ST3GalII and ST3GalIV, respectively) (133). CA 19-9 has been found on various proteins, especially mucins (8,9). In fact, it has been confirmed by *Yue et al.* (2011) that mucins are major carriers of CA 19-9 in the serum of PDAC patients (150). On the other hand, sLeX, an isomer of sLeA, is also upregulated in patients with PDAC (8,37). Moreover, sLeX was found to be elevated in a set of patients who express low levels of sLeA (151). Some authors have linked the presence of inflammatory microenvironment (caused by the presence of inflammatory cytokines) to the elevation of sLeX levels, suggesting that such microenvironments may play a role in the glycosylation of PDAC cells (14,152). This antigen has also been observed on migrating lymphocytes (thus associated with invasion), as well as linked to various proteins associated with PDAC (153,154).

Table 2 Summary of Glycan Alterations in PDAC

Glycan	Change
sLeA/ CA 19-9	Increased; detected by the CA 19-9 assay; found on various protein carriers including mucins.
sLex	Increased; linked to invasion; found on numerous proteins implicated in PDAC.
Tn and sTn antigens	Increased; linked to poor prognosis and metastasis.
Fucosylated and branched N-glycans	Highly branched N-glycans increased in aggressive disease; increased fucosylation; found on numerous proteins implicated in PDAC.
O-GlcNAcylation	Increased; inhibition can reduce tumour growth and progression.
Proteoglycans	Numerous proteoglycans are overexpressed in PDAC (e.g. the heparin sulphate proteoglycan glypican-1 is linked to disease progression and expressed by exosomes).
Galectins	Galectin-1 and Galectin-3 are overexpressed.

Abbreviations: sLeA: sialyl Lewis A antigen; sLeX: sialyl Lewis X antigen; sTn: sialyl Tn antigen.
[Adapted from *Munkley, 2019* (8)].

The overwhelming majority of epithelial cancer cells display truncated O-glycans (155). PDAC is no exception, Tn and sTn) antigens being the most significant truncated O-glycans in this condition (8). Despite not being expressed by the pancreatic tissue under normal circumstances, its levels are elevated in PDAC patients and are often associated with poor

outcome and cancer cell growth and metastasis (8,156–159). Interestingly, some authors have observed that the hypermethylation of a vital chaperone for O-glycan elongation named COSMC (core 1 β3-Gal-T-specific molecular chaperone) was prevalent in PDAC and led to the expression of truncated O-glycans (8,128,158,160).

Aberrant N-glycosylation, as well as N-glycosylation in general, is elevated in PDAC (8,161). Typically, PDAC cells exhibit elevated levels of highly branched N-glycans and altered N-glycan sialylation (particularly α2,3 and α2,6 sialylation) and fucosylation (8,162). Interestingly, a study which employed IMS (imaging mass spectrometry) in order to assess the N-glycome of human PDAC not only confirmed such modifications but observed that N-glycans possessed poly LacNAc (N-Acetyllactosamine) extensions, structures with bisecting GlcNAc residues and terminal GalNAc modifications as well (162). Several proteins involved in PDAC are decorated with N-glycans, some of which include TGF-β (transforming growth factor- β), TNF (tumour necrosis factor), and NF-kB signalling (161). N-glycans may also play a role in influencing the expression of tyrosine kinases and in magnifying the chemosensitivity of drug resistant tumour cells (163).

Another aberration frequently witnessed in PDAC revolves around the increased levels of O-GlcNAcylation (8,14,164). Since O-GlcNAc glycans play a key role in intracellular signalling, its modification leads to changes in the localization and activity of certain metabolic enzymes, histones, and transcriptional regulators (164). For example, O-GlcNAc aberrations observed in transcription factors such as Sp1, β-catenin, SOX2, FOXO3, and YAP stimulate the promotion of gene expression programs that lead to cell proliferation and anti-apoptotic cell states (14,165–169). Aberrant O-GlcNAcylation seems to be associated with the hypoxic environment caused by the dense fibrotic and hypovascular nature of PDAC (14). On one hand, hypoxia increases flux through pathways like glycolysis and glutamine metabolism, converging in the HBP, which is responsible for producing UDP-GlcNAc, a precursor for O-GlcNAcylation (14,170). On the other, hypoxia itself seems to increase the levels of OGT, OGA, O-GlcNAc and the levels of an enzyme named GFPT1 (glutamine–fructose-6-phosphate transaminase 1), which is the first and rate-limiting step of the HBP (14,171).

Proteoglycans are substantially glycosylated proteins which can be either secreted or attached to the cell surface (8). They are bound to GAGs (glycosaminoglycans) such as chondroitin sulphate and heparin sulphate and they are also target of overexpression in PDAC (8,172). One such example is that of glypican-1, a heparin sulphate proteoglycan, which was connected to PDAC progression in mouse models (173–175). Moreover, *Melo et al.* (2015) found glypican-1 to be attached to cancer-derived exosomes present in the serum of PDAC

patients (176). The method that the authors employed was able to distinguish healthy patients and patients suffering from benign pancreatic conditions from early and late-stage PDAC patients (176).

Besides altered glycosylation, cancer cells may also display changes to the expression of proteins that interact with glycans (8). A prime example of this is the overexpression of GAL1 (galectin-1) and GAL3 (galactin-3) in PDAC (177–180). Galectins are a class of lectins, a carbohydrate-binding protein family, and are involved in a number of biological functions which include inflammation, immune response, cell migration, autophagy and signalling (181). They were also recognized to be involved in cancer biology (182). In this particular case, GAL1 seems to be associated with stroma remodelling, cancer cell proliferation, invasion, inflammation, and metastasis, while GAL3 seems to stimulate the production of inflammatory cytokines (178,183,184).

5.3 Biomarkers

A biomarker can be defined as being a biological indicator used to measure with objectivity normal and pathological conditions (185). It can be used to diagnose and classify pathologies, therapy response monitoring, disease risk prediction, and high-risk individual screening (185). As stated in previous sections, PDAC remains a very lethal disease with very poor survival rates. This is in part caused by the fact that it is usually diagnosed at a late stage, which it potentiated by the lack of biomarkers capable of providing a reliable early diagnosis.

Currently, the most widely used serum biomarker for PDAC is CA 19-9 (7–9). It is also the only biomarker for PDAC diagnosis and monitoring approved by the FDA (10,11). In homeostatic conditions, pancreas CA 19-9 is observed on the epithelial surface of the ducts. However, in PDAC, it can be extensively secreted into the lumen of proliferating ducts, subsequently passing into the bloodstream (8). Serum levels of CA 19-9 are mainly used to monitor treatment response and detection of disease recurrence (7–9). Nevertheless, its diagnostic performance is rather limited due to concerns about its specificity and sensitivity (8,9,12,13). In fact, the CA 19-9 assay possesses an approximate sensitivity and specificity of 80% and 85%, respectively. Additionally, the antigen is upregulated not only in PDAC, but in other benign conditions like bile duct obstruction and pancreatitis as well (12–14). Moreover, it has been observed that patients with negative Lewis genotype cannot express CA-19.9. Given that as much as 10% of Caucasians exhibit this phenotype, this biomarker loses further diagnostic power (10,11,15).

Other potential biomarkers have been studied but haven't demonstrated to be viable alternatives to CA 19-9 for early detection of PDAC. Median sensitivity and specificity for CEA (carcinoembryonic antigen) have been placed at 39.5% and 81.3%, respectively. For CA242, the same measurements were placed at 67.8% and 83%, respectively (15,186). However, the combination of CA 19.9 with CA242 did show higher sensitivity (89%), albeit without any impact on specificity (15,186). The combination of CA 19-9 with CEA seems to hold value as a prognostic marker, particularly with regards to advanced disease (187). CA50, CA195, CA72-4, and CA125 were also studied but did not render positive results (15,188).

Given the limitations that current biomarkers possess, there is an urgent need for highly specific circulating biomarkers capable of enabling an earlier detection, more appropriate risk stratification and personalized clinical management (8,9). As described by some studies, glycoconjugates with aberrant glycosylation can often be observed in circulation (8,26,155,159). Therefore, one such strategy could involve targeting aberrant glycans and glycoproteins associated with PDAC (8,125). Below are listed several studies that found numerous glycans and glycoproteins that can be used as biomarkers for the disease and that were detected by lectin-based techniques.

5.3.1 Candidate Novel Biomarkers for Pancreatic Cancer

One strategy for the discovery of novel biomarkers lies on the identification of specific glycan changes in PDAC. To that end, *Yue et al.* (2009) used antibody-lectin sandwich array method to identify glycan changes specific to PDAC. The authors found that MUC16 (mucin-16) was elevated in 65% of cancer patients, while MUC1 (mucin-1) and MUC5AC (mucin-5AC) were only elevated in 30% and 35% of patients, respectively. However, the latter two presented more substantial and diverse glycosylation aberrations. The most common glycan elevations were the TF (Thomsen-Friedenreich) antigen (which corresponds to the core 1 structure described in section 5.1), fucose and Lewis antigens, as well as an unforeseen terminal mannose residues attached to MUC1 and MUC5AC (189).

There have also been numerous studies with the goal of identifying potential single biomarkers for PDAC. Using the lectin SNA (*Sacumbus nigra* agglutinin), *Li et al.* (2009) were able to successfully discriminate PDAC patients from non-cancer patients, by detecting a 69% increase in the A1BG (Alpha-1-β glycoprotein) levels in the previous group (190). Another study used successive analytical techniques, which included MS (mass spectrometry), CZE (capillary zone electrophoresis), and ELLAs (enzyme-linked lectin assays), to analyse the glycosylation of AGP (α-1-acid glycoprotein) from serum samples of 31 individuals. Within

this cohort, there were healthy individuals, chronic pancreatitis patients and PDAC patients. Results indicated an elevation of α1-3 fucosylated glycoforms of AGP when compared to healthy individuals and chronic pancreatitis patients (191). Two other studies that used both lectin microarray and MS found overexpression of syaloglycoproteins such as LAMP1 (lysosome-associated membrane glycoprotein 1) and ORP150 (oxygen-regulated protein 150), and fucosylated and galactosylated glycoproteins, such as CK8 (cytokeratin 8), integrin β1, ICAM1 (intercellular adhesion molecule 1), and RPN2 (ribophorin 2) in certain PDAC cell lines (192,193).

However, another strategy which seems to yield better results than single tumour markers is the employment of a panel of biomarkers. Since CA 19-9 is not elevated in the entirety of PDAC patients, a study profiled the levels of multiple glycans and mucin glycoforms of hundreds of PDAC and other benign pancreatic conditions. Results were further validated by two additional cohorts of patients. The study used both antibodies and lectins in order to capture the glycan structures. The chosen lectin was CCL2 (*Coprinopsis cinerea* lectin 2) due to its specificity for 3' fucose (194). The authors found significant increases in distinct groups of patients in two other glycans: sLeX (both in sulphated in non-sulphated forms) and sialylated type 1 LacNAc. Each biomarker performed as well as CA 19-9 as an individual marker and a panel formed by the 3 glycans performed better than any individual marker. The panel's sensitivity and specificity were both superior to those of the CA 19-9 alone, demonstrating an increased diagnostic accuracy. In the discovery and validation cohorts, these values were, respectively, 85% and 90% for the panel and 54% and 86% for CA 19-9 alone. The numbers were slightly lower for the independent test cohort, reaching 80% and 84% for the panel and 66% and 72% for the CA 19-9 alone (194). Another study which aimed to identify potential biomarkers able to discriminate PDAC from other related conditions, employed lectin extraction and proteomics techniques. First, the authors used a lectin array strategy in order to identify global glycosylation alterations in the collected serum samples. The chosen lectin was AAL (*Aleuria aurantia* lectin), due to its specificity for fucose. ELISA (enzyme-linked immunosorbent assay) and lectin-ELISA were subsequently employed so the potential markers could be validated. The authors found that a biomarker panel made up of CA 19-9, AACT (α-1-antichymotrypsin), THBS1 (thrombospondin-1), and HPT (haptoglobin) surpassed CA 19-9 in distinguishing PDAC patients from non-cancer control groups, reaching an AUC (area under the curve) of 0.95 in conditions without obstructive jaundice. The AUC in conditions with obstructive jaundice was slightly lower, reaching 0.92 (195).

One other interesting approach for early PDAC diagnosis may rest upon the detection of glycans attached to the surface of exosomes. Exosomes are nano-sized extracellular membranous vesicles released into surrounding body fluids that can contain various proteins, genetic components, and, most importantly, glycosylated proteins attached to their membranes (196–198). They may hold diagnostic value owing to their stability, abundant secretion by most cells and their accumulation in the circulation (198). One study carried out by *Choi et al.* (2021) aimed to ascertain the validity of this strategy by employing lectins attached to JNPs (Janus nanoparticles) able to recognize PDAC-derived exosomes isolated from blood samples of PDAC patients and from a culture medium of PDAC cell lines (196). The lectins used by the authors were SNA, Con A (concanavalin A), and AAL. The first was chosen due to its affinity for sialic acid moieties, particularly α2,6 and α2,3 sialic acid, the second due to its affinity for glucose, GlcNAc and mannose moieties, and the third was chosen due to its affinity for fucose moieties (196,199–201). Additionally, the CA19-9 antibody was used for comparison. Not only were the intended exosomes successfully captured by the lectin conjugated JNPs (with comparable affinities to that of the CA 19-9 antibody), but the method was also capable of differentiating metastatic from non-metastatic cells (196). Another example is the lectin microarray carried out by *Sakaue et al*. (2019) which led to the observation that exosomes derived from unresctable PDAC patients possess sialylated forms of CD133, establishing the latter's value as a prognostic biomarker (202).

6 Lectins

Lectins are a diverse group of non-immune proteins that have the ability to recognize and reversibly bind to specific carbohydrates and glycoconjugates without altering the structure of the glycans or the conjugated molecules (31–35). Therefore, they may act as recognition molecules (35). The interaction that these proteins establish with glycans is akin to that which is observed between antigen and antibody or enzyme and substrate (34,36). Moreover, these proteins are distinguished from other glycan-binding macromolecules due to their ability to agglutinate cells (31). One prominent example is that of the phytohemagglutinins, a group of plant lectins that agglutinates red blood cells (203,204). They may exist in free form or attached to cell surfaces (31). Furthermore, biological functions depend on their general properties and location within a given tissue and may include cell cycle regulation, glycoprotein synthesis, innate immunity, roles in protection against infections (31,35). Lectins are of an ubiquitous nature, being expressed in animals, fungi, plants, and bacteria (30,31,35).

6.1 Molecular Structure and Classification

Lectins' specific attachment to certain carbohydrates is conditioned by their three-dimensional structure (and of their binding site in particular), which may vary according to amino acid sequence, metal ion involvement, number of protein subunits and polypeptide nature (31). They may have as many as 12 interaction sites, depending on the molecule's nature and oligomerization state (31,205). Binding site specificity and affinity, as well as the lectin-carbohydrate stability, are determined mostly by hydrogen bonds between the hydroxyl groups belonging to the glycan and amino acid residues in the lectin, and hydrophobic interactions with residues of aromatic amino acids and hydrophobic sections of monosaccharides (31,196,206–208). Binding affinity is variable, but the interaction between glycan and lectin is typically weaker than the interaction between antigen and antibody (209). However, the interaction is stronger between lectins and complex glycans than between lectins and monosaccharides, particularly when this interaction is multivalent (209).

There are several ways to classify lectins. According to overall structure, plant lectins may be divided into merolectins, hololectins, superlectins, and chimerolectins (Figure 6) (209,210). Merolectins are characterized as having one single carbohydrate-binding site and are thus not able to agglutinate (210). Hololectins, on the other hand, possess at least two identical binding sites, conferring them the ability to cause agglutination (210). Superlectins also possess two binding sites, but each has specificity for a different sugar (210,211). Finally,

chimerolectins are essentially fusion proteins constituted by one or more carbohydrate-binding domains fused to a non-related domain, usually with enzymatic activity (210,212,213).

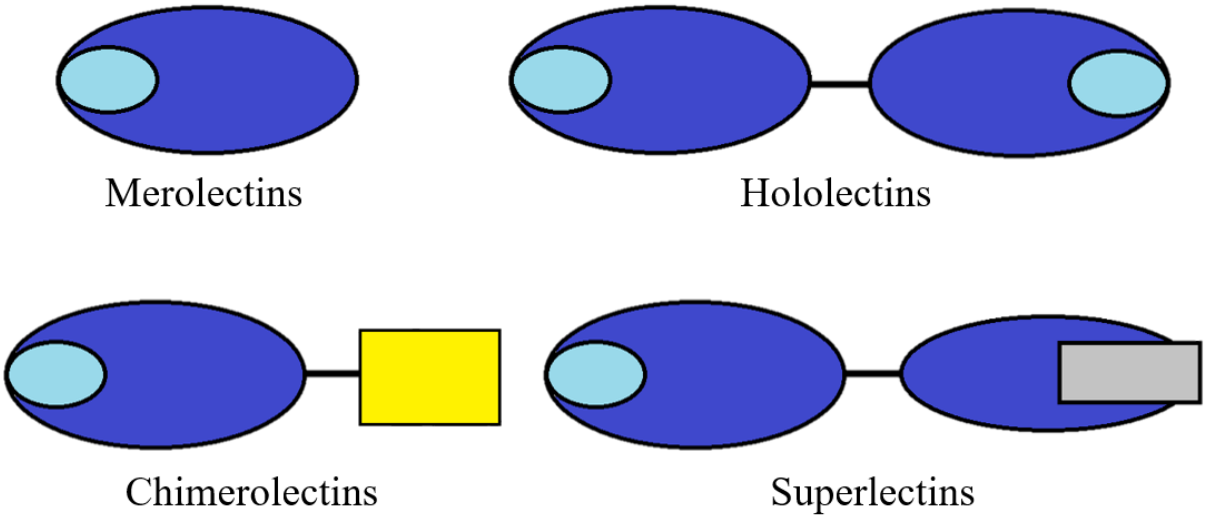

Figure 6 Schematic representation of merolectins, hololectins, superlectins, and chimerolectins.
Lectins are represented by the larger, dark blue elliptical shapes. Their respective binding sites are represented the smaller, light blue elliptical shapes. In the case of superlectins, the grey rectangular shape represents a second binding site with a different carbohydrate specificity. The non-related domain belonging to the chimerolectins is represented by the yellow rectangular shape.
[Adapted from *Van Damme et al., (1998)* (213)]

If structurally and evolutionary related proteins are taken into account, lectins can be classified as amaranthins, chitin-binding lectins, *Cucurbitaceae phloem* lectins, jacalin-related lectins, legume lectins, monocot mannose binding lectins, and type-2 ribosome inactivating proteins (RIP) (31,210). Lectins can also be classified according to their biological function, being divided into hemagglutinins, adhesins, selectins, galectins, or siglecs (214). However, in the context of identifying new glycosylated biomarkers, one of the most valuable to classified lectins is to divide them according to their highest specificity towards a given monosaccharide structure (215). They are thus divided into five groups: mannose and glucose-binding, galactose and GalNAc-binding, GlcNAc-binding, fucose-binding, and N-acetylneuraminic acid-binding lectins (215). There could be affinity for other monosaccharides, but it is found rarely (215).

6.2 Application of Lectins in the Diagnosis of Pancreatic Cancer

As discussed above, several studies throughout the years have been focusing on glycosylation aberrations observed in PDAC and their potential use as biomarkers for diagnosis, screening, and prognosis of this disease. The main observed aberrations include increased sialylation (particularly α2,3 and α2,6 sialylation), elevation of the sialyl Lewis antigensA and X (sLeA and sLeX) levels, increased truncated O-glycans (Tn and sTn antigens), increased fucosylated and aberrantly branched N-glycans, upregulation of specific proteoglycans and galectins, and increased O-GlcNAcylation (8,14,147–149). Moreover, lectins have been used

in the last few decades to identify potential novel biomarkers for PDAC because of their reversible and, most importantly, specific attachment to certain glycans without adulterating their structure (37,151,185,189–192,195,196,202).

Table 3 summarizes the glycan specificities of lectins which have been used in the identification on glycosylation aberrations in PDAC throughout the years.

Table 3 Glycan specificity of lectins used in the detection of glycosylation aberrations in PDAC

Lectins	Glycan specificity	References
AAL	α1,3/α1,4 and α1,6 fucosylations	(192,195,196,200,201)
BPL	Galβ1,3GalNAc	(185,200)
CCL2	Fucα1,3GlcNAc	(217)
Con A	α-Man > α-Glc > GlcNAc	(196,200)
DBA	α-Linked GalNAc	(189,192)
ECL	Galβ4GlcNAc	(185)
GSL-I	αGalNAc, GalNAcα-Thr/Ser, and αGal	(189)
GSL-II	Agalactosylated tri/tetra antennary glycans, GlcNAc	(185)
Jacalin	Galβ1,3GalNAcα-Thr/ Ser (TF), GalNAcα-Thr/ Ser (Tn)	(189)
LCA	Fucα1,6GlcNAc and αMan	(189,190)
LEL	$(GlcNAc)_n$ and poly(LacNAc) (2–3 consecutive)	(189)
MAL	Terminal sialic acid, NeuAc-Gal-GlcNAc with sialic acid at the 3 position of the GalNAc residue	(190)
PHA-L	Tri/tetra-antennary complex-type N-glycan	(185)
PhoSL	Fucα1,6	(191,218)
PNA	Galβ3GalNAc	(185)
PTL	Fucα1,6	(200)
RCA-I	Galβ4GlcNAc	(185)
RSL	α-fucosylation, all linkages	(151)
SBA	GalNAc, GalNAcα3Gal	(185)
SNA	α2,6 (major) and α2,3 sialic acid (minor)	(189,190,196,199)
SRL	Terminal GlcNAc	(151)
STL	GlcNAcβ1-4GlcNAc	(202)
UDA	GlcNAcβ1-4GlcNAc, Man	(202)
UEA	Terminal or subterminal Fucα1,2Gal	(189,192)
VVL	αGalNAc, GalNAcα3Gal	(185)
WGA	GlcNAcβ1,4GlcNAc or lactosamine > sialic acid	(189)

Abbreviations: AAL - *Aleuria aurantia* lectin; BPL - *Bauhinia purpurea* lectin; CCL2 - *Coprinopsis cinerea* lectin 2; Con A - Concanavalin A; DBA - *Dolichos biflorus* agglutinin; ECL - *Erythrina cristagalli* lectin; GSL-I - *Griffonia simplicifolia* lectin I; GSL-II - *Griffonia simplicifolia* lectin II; LCA - *Lens culinaris* agglutinin; LEL - *Lycopersicon esculentum* lectin; MAL - *Maackia amurensis* lectin II; PHA-L - *Phaseolus vulgaris* agglutinin-L; PhoSL - *Pholiota squarrosa* lectin; PNA - Peanut agglutinin; PTL - *Pinellia ternata* lectin; RCA-I - *Ricinus communis* agglutinin; RSL - *Ralstonia solanacearum* lectin; SBA -

Soybean agglutinin; SNA - *Sambucus nigra* lectin; SRL - *Sclerotia rolfsii* lectin; STL - *Solanum tuberosum* lectin; UDA - *Urtica dioica* lectin; UEA - *Ulex europaeus* agglutinin; VVL - *Vicia villosa* lectin; WGA - Wheat germ agglutinin.

Furthermore, lectins have been shown to display a good performance in *in vitro* assays using small amounts of sample, susceptibility to recombinant production, and the required precision and throughput for biomarker studies (37–39). Some of these techniques include ELLA, immobilized-lectin affinity chromatography, lectin histochemistry, lectin blotting, and lectin array (185,216).

ELLA was introduced in the 1980s and it is similar in nature to ELISA, only it uses lectins instead of antibodies (216,219). It may have one of three approaches: direct assay, hybrid assay, and sandwich enzyme-linked lectin assay, as illustrated in Figure 7 (216). For all three, glycoproteins and glycans are most often detected using a lectin that is conjugated to an enzyme that converts a colourless substrate into a coloured product (216). The intensity of said coloration is measured by a spectrophotometer, which allows the determination of the coated glycoconjugates levels' (216).

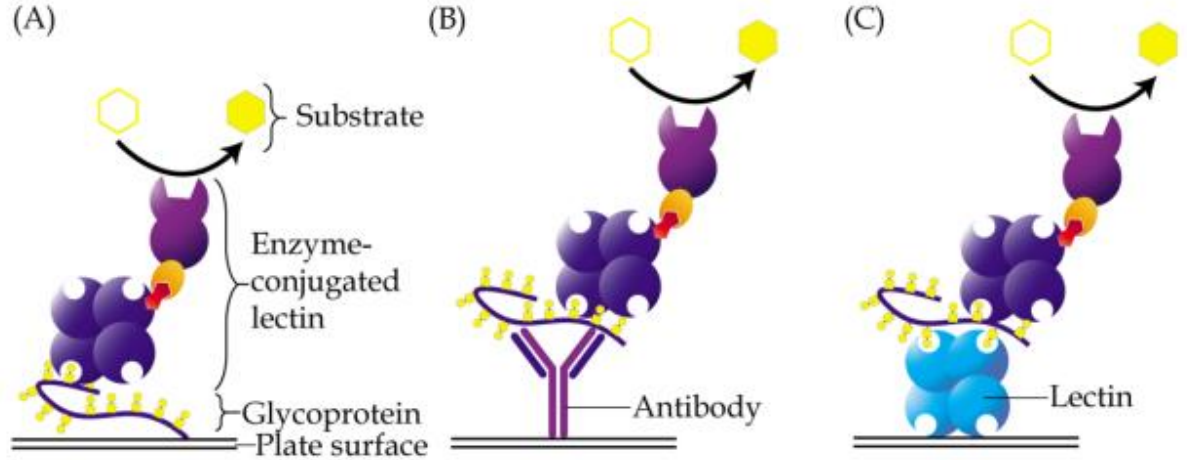

Figure 7 Different approaches of enzyme-linked lectin assay.
(A) Direct assay: samples are directly coated on the surface of a microtiter plate, followed by the addition of an enzyme-conjugated lectin. (B) Hybrid assay: an antibody is coated onto the plate, capturing specific glycoproteins. Afterwards, the enzyme-conjugated lectin is added. (C) Sandwich enzyme-linked lectin assay: it involves two distinct lectins. The first is coated onto the plate and used to capture the glycoprotein of interest. The second is used as the detection reagent.
[Adapted from *Hashim et al.* (2017) (216)]

Immobilized-lectin affinity chromatography is based on the affinity between certain glycoproteins and a given lectin (Figure 8) (216,220). It involves the introduction of the sample (usually a bodily fluid) into a chromatography column packed with a gel matrix conjugated with a specific lectin. As the sample runs through the column, non-binding proteins are washed out while binding glycoproteins are captured by the lectins. The lectin-bound glycoproteins are then eluted with certain carbohydrate solutions and identified with proteomics. The technique is frequently supplemented by spectrometry analysis (216).

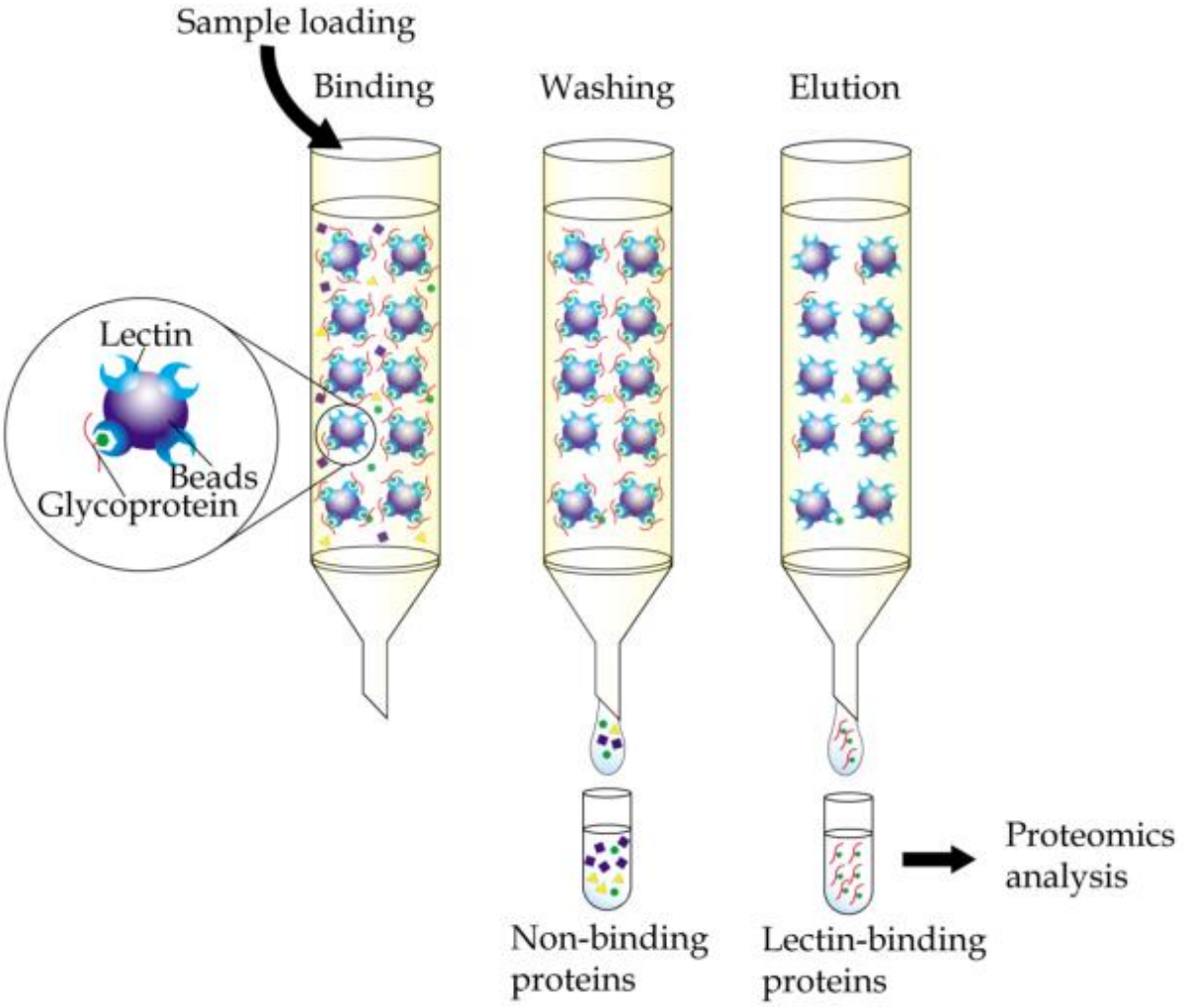

Figure 8 Workflow of immobilized-lectin affinity chromatography.
Potential biomarkers may be identified by running samples from cancer patients through a affinity column packed with lectin conjugated gel matrix. Non-binding proteins are washed out, while the bound glycoproteins are eluted using specific carbohydrate solutions. The lectin-bound glycoproteins are, usually, then identified through proteomics analysis.
[Adapted from *Hashim et al., 2017* (216)].

Lectin histochemistry is a microscopy technique that uses lectins in order to identify and map carbohydrate structures within the tissues (216,221). It can be done directly (generally less sensitive) or indirectly (222). The direct method involves the direct linkage of lectins to fluorophores, colloidal gold, enzymes or ferritin, depending on the technique (222). The indirect method involves the conjugating the lectins to biotin or digoxigenin (222). The detection is then obtained with enzyme-linked streptavidin or anti-digoxigenin, respectively (222).

Lectin blotting follows the basis of western blotting. Thus, samples are resolved using polyacrylamide gel electrophoresis and subsequently transferred onto a nitrocellulose or PVDF (polyvinylidene fluoride) membrane (223). However, glycan-detecting lectins are used as probes instead (216). Much like histochemistry, visualization is made possible by conjugates, which include enzymes, radioactive isotopes, colloidal gold, digoxigenin, biotin, and fluorescent dyes (216). Nevertheless, lectin concentration should be optimal in order to reduce

false positive binding (216). Tough powerful, this tool is not considered suitable for routine diagnosis, having been used, in cancer biomarker studies, for comprehensive profiling of glycoproteins in bodily fluids (216).

Lectin array is a technique designed to analyse glycans swiftly and with high sensitivity and throughput (216). It achieves this by employing several lectins simultaneously which are immobilized onto a solid support in a spatially dense way with the purpose of detecting different glycan content from several glycoconjugates belonging to a single sample (216,224,225). The main material used for the solid support is a glass slide and lectins are coated to such surface either by physical adsorption or covalent bonding (216). The lectin droplets are arrayed on the glass in a grid pattern in such a way that a single lectin occupies a single spot (216). Samples are usually labelled beforehand with fluorophore or chromophore. After binding to lectins, the labelled glycoproteins emit fluorescence when a suitable scanner is employed (216).

7 Conclusions

PDAC remains a lethal disease with dismal prognosis, grave consequences for patients' quality of life and rising incidence and death rates. It is characterized by displaying little to no symptoms (and often very unspecific) in its early stages, which generally represents the only time where treatment would be possible. Moreover, there are currently no biomarkers being used in the clinical practice which allow an early diagnosis. Therefore, the need for the development of novel diagnostic and screening protocols capable of providing an early detection, more appropriate risk stratification and personalized clinical management is urgent.

Given that PDAC patients experience very characteristic glycosylation aberrations and given that lectins specifically bind to carbohydrate structures, the use of these proteins presents itself as a viable and valuable avenue to identify novel potential biomarkers. In fact, several individual biomarkers identified through the use of lectins have already been documented, namely A1BG, α1-3 fucosylated glycoforms of AGP, LAMP1, ORP150, CK8, integrin β1, ICAM1, and RPN2. Another interesting and promising approach which has been documented is the detection of aberrant glycans attached to the surface of exosomes secreted into the bloodstream of PDAC patients.

However, the most auspicious approach seems to be the employment of a panel of multiple biomarkers instead of a single one. Some of the panels which have been proposed include a panel constituted by CA 19-9, sLeX variants and sialylated type 1 LacNAc and another panel constituted by CA 19-9, AACT, THBS1, and HPT. Both panels demonstrated a superior diagnostic performance than that of the CA 19-9 alone. It is also important to add that the lectins used to identify these panels (CCL2 and AAL, respectively) can be classified as fucose-binding lectins. Although these panels deserve further investigation for its diagnostic, screening and prognostic potential, the results seem to be very promising.

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