Diabetes Mellitus and Reprogrammed Glucose Metabolism in Pancreatic Cancer: Features for Clinical Translation

Daniela Basso*, Andrea Padoan, Paola Fogar, Carlo-Federico Zambon and Mario Plebani
Department of Medicine–DIMED, University of Padova, Italy

Abstract
The reprogrammed metabolism of cancer cells underlies the shift of glucose energetics from the highly efficient oxidative phosphorylation to the less efficient aerobic glycolysis, the Warburg effect. This phenomenon, with the activation of the glutamine pathway, advantages survival and proliferation of pancreatic ductal adenocarcinoma (PDAC) cells, which live in an adverse hypoxic and nutrient restricted microenvironment. In PDAC, glucose metabolic alterations occur also at the whole organism, Diabetes Mellitus (DM) being diagnosed in approximately 60% to 80% of patients. The association between PDAC and DM is a dual face phenomenon, DM being both a risk factor for and a consequence of this tumor type. Data from epidemiology indicate that longstanding DM increases PDAC risk 1.5 to 2.0 fold, probably because of the pro-proliferative effects of hyperinsulinemia. By contrast early onset DM, i.e. diabetes diagnosed no more than two years prior to cancer diagnosis, is considered a consequence of PDAC. Secondary DM is due to complex interactions between tumor cells, tumor microenvironment and pancreatic endocrine cells. In this scenario the role of the inflammatory S100A8 calcium binding protein, matrix metalloproteinases, Vanin 1 or amylin has been experimentally demonstrated. However, the efforts made to translate in the clinical practice any individual new potential biomarker failed, because none reached enough sensitivity and specificity to be considered a reliable biomarker to diagnose PDAC even in high risk subjects as those with new onset DM. Therefore the identification and clinical validation of new biomarkers remains a challenge for future studies.

Introduction
The glucose metabolism alterations present at both the cancer cell site and throughout the organism level in cancer patients are particularly evident in pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer related deaths [1]. Glucose transporter GLUT1 over expression at the cancer cell site favours the uptake of glucose, the main source for cellular energetics, on which in PET imaging the use of the tracer \(^{18}\)fluorodeoxyglucose is based [2]. In cancer cells glucose metabolism is reprogrammed and, even in the presence of oxygen, glucose is mainly processed in the cytosol to pyruvate, which largely escapes from the energy efficient Krebs cycle in the mitochondria [3]. This phenomenon, first described by Otto Warburg almost 100 years ago, and now known as Warburg's effect or "aerobic glycolysis", is considered one of the emerging hallmarks of cancer [4]. The clinical manifestation of altered glucose metabolism in the organism is diabetes mellitus, considered a risk factor for, and a consequence of, PDAC [5]. Although it is not known whether glucose metabolic alterations in the cancer cell, and in the entire organism, influence each other, it has been suggested that insulin and insulin-like growth factors play a part in cancer onset and progression [6,7].

Alterations in glucose metabolism at the cancer cell site
Although first described almost 100 years ago, renewed attention in the Warburg effect over the last few decades, has led to the definition of two main concepts:

1. Metabolic reprogramming is a feature of cancer cells contributing to proliferation and metastases [3,8];
2. Drugs targeting cancer metabolism might enhance the efficacy of chemotherapy [9].

In PDAC, cancer cells are dispersed within a hypovascular dense desmoplasia, which contributes to a hypoxic and nutrient deficient tumoral microenvironment. These features might limit the access of cancer cells to fuel and nutrients, indispensable for the biosynthesis of amino acids and nucleotides, required for cell proliferation. By reprogramming their metabolism, PDAC cells are...
enabled to support amino acids and nucleotide biosynthesis, thus
deriving advantage from the adverse microenvironment. In cancer
cells glucose uptake and glycolysis are favoured by the over expression
of the glucose transporter GLUT1 and of a series of glycolytic
enzymes, including lactate dehydrogenase (LDH, that converts
pyruvate into lactate), hexokinase 2 (HK2, the first rate limiting
enzyme of glycolysis) and pyruvate kinase M2 (PKM2, the final rate
limiting enzyme of glycolysis) [2]. Even in the presence of oxygen,
only a minimal part of pyruvate enters mitochondrial oxidative
phosphorylation (OXPHOS), mainly being converted to lactate; this
is due, at least in part, to the inactivation of pyruvate dehydrogenase
(PDH, which converts pyruvate into acetyl-CoA for the TCA cycle).
Lactate accumulates in the microenvironment and lowers pH, which
induces the expression of matrix metalloproteinases, mainly MMP-
2 and MMP-9 [3], while inhibiting the immune response [10], thus
favouring the metastatic potential. Aerobic glycolysis is less efficient
than OXPHOS in terms of energy supply: only four rather than
36 ATP moles per mole of glucose are produced. Energy supply by
OXPHOS in cancer cells may be supported by the glutamine
pathway, which also supports the biosynthesis of nucleotides, lipids
and glutathione [11]. The metabolic reprogramming of cancer cells by
means of aerobic glycolysis and glutaminolysis, appears to be closely
correlated with the genetic landscape of cancer cells themselves. KRAS
activating mutations, TP53 loss of function and MYC over expression,
frequently found in PDAC, regulate the Warburg’s effect [2,9]. It
has recently emerged that in tumours metabolic reprogramming is
not restricted to cancer cells; this phenomenon, also known as the
reverse Warburg effect, also involves stromal cells, such as cancer
associated fibroblasts (CAFs). In pseudo-hypoxyc conditions, CAFs
produce HIF-1 alpha, which promotes glycolysis with the production
of lactate that further reduces pH, and glutamate, which might fuel
cancer cells. This metabolic symbiosis also occurs between cancer cells
and cancer stem cells [3], and between cancer cells and immune cells
[12]. Intriguingly, elsewhere we observed a reverse Warburg effect in
myoblasts, the magnitude of lactate production being correlated with
PDAC-associated diabetes mellitus, suggesting that there is a link
between alterations in glucose metabolism at the cancer cell site and
in the whole organism [13].

**Alterations in Glucose Metabolism in the Whole Organism**

**Diabetes mellitus as a cause of PDAC – evidence from epidemiology**

The association between diabetes mellitus and PDAC has been
recognised for over 100 years. Diabetes mellitus or reduced glucose
tolerance are diagnosed in the majority of PDAC patients, i.e. 50% and
30-40% of cases, respectively [14]. This high association rate
was soon to give rise to the question as to whether diabetes mellitus
was the cause or effect of PDAC. Epidemiological and experimental

---

**Table 1: PDAC prevalence impacts on the positive (PPV) and negative (NPV) predictive values of biomarkers.**

<table>
<thead>
<tr>
<th>Disease prevalence</th>
<th>Biomarker Sensitivity</th>
<th>Biomarker Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00013</td>
<td>0.90</td>
<td>0.90</td>
<td>0.0012</td>
<td>0.9999</td>
</tr>
<tr>
<td>0.03</td>
<td>0.90</td>
<td>0.90</td>
<td>0.2177</td>
<td>0.9966</td>
</tr>
<tr>
<td>0.00013</td>
<td>0.95</td>
<td>0.95</td>
<td>0.0025</td>
<td>0.9999</td>
</tr>
<tr>
<td>0.03</td>
<td>0.95</td>
<td>0.95</td>
<td>0.3701</td>
<td>0.9984</td>
</tr>
<tr>
<td>0.00013</td>
<td>0.99</td>
<td>0.99</td>
<td>0.0129</td>
<td>0.9999</td>
</tr>
<tr>
<td>0.03</td>
<td>0.99</td>
<td>0.99</td>
<td>0.7538</td>
<td>0.9997</td>
</tr>
</tbody>
</table>
findings from the Mayo Clinic demonstrating a progressive increase in blood glucose starting from 36 months prior to PDAC diagnosis [26], thus supporting the proposal of screening asymptomatic individuals using new-onset hyperglycemia and diabetes as a first filter to detect those at a higher risk of PDAC. However this detection calls for a reliable biomarker of pancreatic cancer-associated diabetes mellitus [27].

Longstanding diabetes mellitus preceding PDAC is not a single entity, but a highly complex and heterogeneous disease. The heterogeneity is due to differences in comorbidities, medications, compensation, and in some cases, exposure to diabetogenic and/or carcinogenic environmental factors. The metabolic syndrome, the main comorbidity of diabetes mellitus to have been investigated, is a complex of inter-related co-existing conditions, mainly insulin resistance and diabetes, hypertension, dyslipidemia and obesity. Epidemiological studies exploring the role of diabetes mellitus as a risk factor for PDAC while taking other components of the metabolic syndrome into account have confirmed that diabetes mellitus has a carcinogenetic environmental factor. The metabolic syndrome, the syndrome components appear to enhance the risk of PDAC [29].

Epidemiological studies exploring the role of diabetes mellitus as a risk factor for PDAC since chronic hyperglycemia can induce an increased tumour cell proliferation and migration by enhancing the release of the chemokine CXCL12 from stromal pancreatic stellate cells [32]. Although the specific aim of these studies was not to investigate how poor glycemic control impacts on PDAC risk, in a population of male smokers fasting glucose was shown to correlate with PDAC risk [33], and in their dose response meta-analysis Liao et al. [34] found a linear dose-response relation between fasting blood glucose concentration and the rate of PDAC, every 0.56 mmol/L increase in fasting blood glucose being associated with a 14% increase in the rate of pancreatic cancer. Fasting glucose is, however, an imperfect index of the glycemic control, glycated haemoglobin (HbA1c) being a much more reliable tracer of long-term glucose exposure. The pre-diagnostic levels of HbA1c are also positively correlated with PDAC risk [30]. A poor glycemic control in diabetics might be regarded as a potentially relevant risk factor for PDAC since chronic hyperglycemia can induce an increased tumour cell proliferation and migration by enhancing the release of the chemokine CXCL12 from stromal pancreatic stellate cells [32]. Although the specific aim of these studies was not to investigate how poor glycemic control impacts on PDAC risk, in a population of male smokers fasting glucose was shown to correlate with PDAC risk [33], and in their dose response meta-analysis Liao et al. [34] found a linear dose-response relation between fasting blood glucose concentration and the rate of PDAC, every 0.56 mmol/L increase in fasting blood glucose being associated with a 14% increase in the rate of pancreatic cancer. Fasting glucose is, however, an imperfect index of the glycemic control, glycated haemoglobin (HbA1c) being a much more reliable tracer of long-term glucose exposure. The pre-diagnostic levels of HbA1c are also positively correlated with PDAC risk [30].

Although the specific aim of these studies was not to investigate how poor glycemic control impacts on PDAC risk, in a population of male smokers fasting glucose was shown to correlate with PDAC risk [33], and in their dose response meta-analysis Liao et al. [34] found a linear dose-response relation between fasting blood glucose concentration and the rate of PDAC, every 0.56 mmol/L increase in fasting blood glucose being associated with a 14% increase in the rate of pancreatic cancer. Fasting glucose is, however, an imperfect index of the glycemic control, glycated haemoglobin (HbA1c) being a much more reliable tracer of long-term glucose exposure. The pre-diagnostic levels of HbA1c are also positively correlated with PDAC risk [30].

The rate of pancreatic cancer. Fasting glucose is, however, an imperfect index of the glycemic control, glycated haemoglobin (HbA1c) being a much more reliable tracer of long-term glucose exposure. The pre-diagnostic levels of HbA1c are also positively correlated with PDAC risk [30].

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Positive/Total PDAC cases (Sensitivity)</th>
<th>Negative/Total diabetes cases (Specificity%)</th>
<th>Negative/Total controls (Specificity%)</th>
<th>Reference Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 19-9</td>
<td>39/68 (57%) PDAC with new onset DM</td>
<td>181/2295 (79%) new onset DM</td>
<td>NA</td>
<td>[57]</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>43/80 (54%) PDAC with new onset DM</td>
<td>78/85 (92%) new onset DM</td>
<td>76/80 (95%)</td>
<td>[58]</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>47/60 (78%) PDAC with DM</td>
<td>42/43 (98%) Type 2 DM</td>
<td>29/30 (97%)</td>
<td>[40]</td>
</tr>
<tr>
<td>Plasma IAPP</td>
<td>17/30 (57%)</td>
<td>23/23 (100%)</td>
<td>24/25 (96%)</td>
<td>[59]</td>
</tr>
<tr>
<td>Plasma IAPP</td>
<td>22/60 (36%) PDAC with DM</td>
<td>8/9 (89%)</td>
<td>104/107 (97%)</td>
<td>[60]</td>
</tr>
<tr>
<td>Combined blood mRNA expression of vanin 1 (VNN1) and matrix metalloproteinase 9 (MMP9)</td>
<td>23/24 (96%) PDAC with DM</td>
<td>NA</td>
<td>19/25 (76%)</td>
<td>[61]</td>
</tr>
<tr>
<td>Blood mRNA expression of MMP9</td>
<td>31/60 (52%) PDAC with DM</td>
<td>37/43 (86%) Type 2 DM</td>
<td>30/30 (100%)</td>
<td>[40]</td>
</tr>
<tr>
<td>Serum cysteamine (downstream molecules of VNN1)</td>
<td>8/18 (44%) PDAC with new onset DM</td>
<td>13/15 (87%) new onset DM</td>
<td>15/15 (100%)</td>
<td>[54]</td>
</tr>
<tr>
<td>Plasma adrenomedullin</td>
<td>16/30 (53%) PDAC with new onset DM</td>
<td>24/27 (89%) new onset DM</td>
<td>25/28 (89%)</td>
<td>[52]</td>
</tr>
<tr>
<td>Combined serum miR-483-5p, miR-19a, miR-29a, miR-20a, miR-24, and miR-25</td>
<td>47/50 (95%) PDAC with new onset DM</td>
<td>30/50 (60%) new onset DM</td>
<td>30/50 (60%)</td>
<td>[58]</td>
</tr>
<tr>
<td>Combined model with CA 19-9, Apolipoprotein A1 and complement C3</td>
<td>51/57 (90%) PDAC with DM</td>
<td>54/68 (80%) Chronic pancreatitis with DM (n=17) and Type 2 DM (n=51)</td>
<td>NA</td>
<td>[62]</td>
</tr>
</tbody>
</table>

Table 2: Proposed biomarkers for the diagnosis of PDAC in the selected population of patients with diabetes mellitus.
Diabetes mellitus as a consequence of PDAC – clinical and experimental evidence

The concept that early onset diabetes mellitus is a consequence of PDAC is supported not only by the epidemiological observations described in the previous section, but also by the clinical observation that overt diabetes mellitus or reduced glucose tolerance is found in more than 60% of patients at PDAC diagnosis [38–40], and that diabetes mellitus ameliorates after surgical removal of the tumor [41,42]. This last finding, furthermore, argues against the simple hypothesis that pancreatic cancer-associated diabetes mellitus is due to cancer-related islet cells destruction and supports the hypothesis that PDAC induces diabetes through the release of diabetogenic molecules, which might cause peripheral insulin resistance and/or impaired insulin release from beta-cells, both of which have been found in PDAC patients [43–45]. Another clinical issue concerns the impact of diabetes mellitus on the prognosis of patients with PDAC. Fasting glucose levels are positively associated with the overall cancer-related mortality [46], and survival after surgical removal of PDAC was shown to be significantly affected by uncontrolled longstanding severe hyperglycemia [47].

PDAC-associated diabetes mellitus and islet cell dysfunction

Several research groups, ours included, have thrown light on the pathophysiological mechanisms underlying PDAC-associated diabetes mellitus, which is due to a complex interplay between tumor and stromal-derived molecules, pancreatic endocrine cells and insulin targeted peripheral tissues/organs. The key player molecules in this process appear to be matrix metalloproteinases and the calcium binding protein, S100A8, a 10 kDa protein belonging to the family of S100 Ca2+ binding EF hand type proteins [48], which form homo- and hetero-complexes, S100A9 being the main binding partner of S100A8. The resulting S100A8/A9 heterodimer, also known as calprotectin, is normally produced and released by polymorphonuclear and mononuclear cells. The extracellular S100A8/A9 complex acts as a ligand for different receptors, including RAGE and TLR4. In PDAC, high S100A8 expression is found in the stromal compartment when tumor cells express the tumor suppressor gene SMAD4, while, when SMAD4 is lost, S100A8 is no longer expressed by stromal cells, but by cancer cells [49]. This inverse relationship between SMAD4 and S100A8 expression is further supported by findings made “in vitro”: when pancreatic cancer cells without SMAD4 expression, but with S100A8 expression are forced to express SMAD4 by transfection, they lose their ability to express S100A8 [50]. The numerous biological effects of S100A8 in PDAC include epithelial to mesenchymal transition and the SMAD4-dependent inhibition, or activation, of pro-survival and pro-metastatic intracellular signalling pathways such as NF-κB, AKT and mTOR [51]. But S100A8 can also induce the expression of MMP8 and of MMP9 by inflammatory mononuclear cells [40]. Intriguingly, S100A8 is a substrate for metalloproteinases, which catalyse the release of the N-terminal 14 aminocid peptide from the entire molecule; this, in turn, alters intracellular calcium fluxes and renders beta-cells insensitive to glucose stimulation, leading to a reduced insulin secretion, a potential cause of PDAC-associated diabetes mellitus [40,50]. It has also been demonstrated that glucose stimulated insulin secretion is reduced by adrenomedullin, a pluripotent hormone overexpressed in PDAC [52]. This hormone shares homology with amylin or islet amyloid polypeptide (IAPP), which is co-secreted with insulin by beta-cells at a constant ratio in the normal pancreas, while in the presence of PDAC-conditioned media, the IAPP/insulin molar ratio increases [53]. IAPP has also been found to reduce arginine stimulated insulin, glucagon and somatostatin release, and might play a part in determining islet dysfunction in PDAC patients [43]. It has been observed that beta-cell proliferation impairment with apoptosis induction is dependent on the enzyme overexpressed in PDAC, vanin 1 (VNN1), which hydrolyzes pantetheine and produces Vitamin B5 and cysteamine [54].

PDAC-associated diabetes mellitus and impaired glucose metabolism in peripheral tissues

Muscle, liver and fat cells are the principal targets of insulin and glucagon, the two main hormones regulating glucose homeostasis. By binding its receptor, insulin favours glucose entry and storage as glycogen in target cells, while glucagon, the counter regulatory hormone, has the opposite effect, inducing glycoegenolysis and glucose extrusion. The Insulin Receptor (IR), a tetrameric structure made up of two alpha and two beta subunits, binds insulin through its alpha chains and triggers intracellular signalling by the tyrosine kinase activity of its beta chains. The analysis of the IR signalling cascade in skeletal muscle tissue from PDAC patients has demonstrated that insulin binding, tirosin kinase activity of the IR and the content of the insulin receptor binding substrate 1 (IRS1) did not change with respect to control tissue samples, while the phosphatidylinositol 3-kinase (PI3-K) activity, glucose transport and glycoegen synthease activity were impaired in pancreatic cancer patients [43,45]. We demonstrated that pancreatic cancer cells impair glycosylation of both muscle and liver cells through the activity of a low molecular weight tumor product that favours the metabolic shift of glucose from oxidative phosphorylation to aerobic glycolysis (lactate accumulation) and, in liver cells, to triglyceride biosynthesis through the accumulation of the intermediate D-1,2-diacylglycerol [13,55].

Biomarkers of PDAC-associated diabetes mellitus

Experimental studies have been performed to verify the pathophysiology of PDAC-associated diabetes mellitus and to identify any potential tumor derived molecule involved in causing islet cell and or peripheral glucose metabolic alterations, the end point being to identify a biomarker able to distinguish between the presence or absence of PDAC in patients with new onset diabetes mellitus. When exploring emerging biomarkers in this setting, a careful consideration should be made of the disease prevalence, since it significantly impacts on positive and negative predictive values for any given combination of sensitivity and specificity of the studied biomarker. The prevalence of PDAC varies between unselected and selected populations: in the whole asymptomatic population, its prevalence appears almost equal to its incidence (13/100000 per year, 0.013%) [56], being 230 fold lower than that reported among the selected population of asymptomatic patients with diabetes mellitus (almost 3%) [57]. Based on prevalence, the positive and negative predictive values of a biomarker with a given sensitivity and specificity are reported in Table 1. PDAC prevalence impacts on the positive (PPV) and negative (NPV) predictive values of biomarkers. PDAC prevalence in the general population (0.00013%) and among patients with new onset diabetes mellitus (0.03) were considered to calculate PPV and NPV of biomarkers with 90, 95 and 99% sensitivity and specificity. Positive predictive value (NPV): Sensitivity x (1-Prevalence)/Specificity (1-Prevalence) + (1-Sensitivity) x Prevalence. Positive predictive value (PPV): Sensitivity x Prevalence/ Sensitivity x Prevalence + (1-Specificity) x (1-Prevalence) Clearly, in
the unselected general population, positive findings of a biomarker with a very high sensitivity and specificity (99%) are due to PDAC in about 1/100 cases, thus supporting the notion that PDAC screening of the general population is not recommended. By contrast, in selected patients, a biomarker with a sensitivity and specificity of 95% could allow the identification of a potentially relevant number of cases, i.e. 37 PDAC out of 100 patients with positive results. It is also clear that sensitivity and specificity should be at least 90% to limit over-diagnosis and over-use of invasive diagnostic procedures. The biomarkers suggested for diagnosing PDAC in the selected population of patients with new-onset diabetes mellitus, include the established CA 19-9 marker as well as emerging new potential biomarkers, such as proteins, peptides, microRNA and mRNA, the detailed description of which also in terms of sensitivity and specificity is reported in Table 2. Overall none of the proposed biomarkers is superior to the established marker CA 19-9 in terms of sensitivity and specificity for PDAC diagnosis in patients with diabetes mellitus. Moreover, since none attain a sensitivity and specificity of at least 90%, their use cannot be supported in clinical practice.

In conclusion, the efforts made to translate in the clinical practice new potential biomarkers of PDAC-associated diabetes mellitus have failed, due to low sensitivity and specificity. Therefore the identification and clinical validation of new biomarkers remains a challenge for future studies.

References


