Translational Pancreatology. New Approaches in the Development of Novel Biomarkers as Screening Methodologies for Pancreatic Cancer.

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Abstract

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the rare cancers for which no significant improvements in diagnosis and therapy have been made in the last 30 years. Despite considerable progress the median survival of most patients is 5 to 6 months. Currently, 80% of patients diagnosed with pancreatic adenocarcinoma present with locally invasive and/or metastatic disease, resulting in a poor 5-year survival of <5%. Hence, less than 20% of patients with PDAC undergo potentially curative surgery, whereas the remaining patients, can only be offered palliative treatment. Only 15–20% of patients undergo resection.

The development of a diagnostic tool for early detection of patients with this malignancy may significantly impact their prognosis. Pancreatic cancer has a strong correlation between tumor stage and prognosis. The size of the tumors is also important in a general sense, the smaller ones have metastases less frequently than the larger ones at the time of diagnosis and, therefore, are more likely to be cured by surgery. Therefore, it is evident that the earliest detection of cancers is one of the keys to reducing deaths from these diseases and this includes the cancer of the pancreas with its high mortality rates. However, effective early diagnosis remains difficult and depends mainly on imaging modalities and the development of screening methodologies with highly sensitive and specific biomarkers.

This review summarizes recent advances in effective screening for early diagnosis of PDAC using novel molecular biomarkers discovered from various studies including protein biomarkers, mutant DNA templates identified in the circulation in blood samples (ctDNA), DNA promoter hypermethylation and measurement of microRNA as other possible noninvasive biomarker for early detection of Pancreatic Cancer.

These data have high translational relevance and suggest that early detection is the key issue for improving the prognosis of this aggressive disease. Although many biomarkers for early detection of Pancreatic Cancer (PC) have been discovered through various methods, effective panels of noninvasive biomarkers for screening and early detection of Pancreatic Ductal Adenocarcinoma (PDAC) are not available yet.

The increased number of translational studies would be able to provide this remarkable diagnostic tool in a near future. Nevertheless, larger scale and rigorous validation is required before their application in the clinic.

Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is the predominant histologic type of pancreatic cancer (PC) and has one of the worse prognoses of all types of cancer leading to 227,000 deaths annually worldwide. It is currently the fourth leading cause of cancer-related death in the world. The majority of patients with PC progress to either locally advanced or metastatic disease in the asymptomatic phase, as many as 80% presents late with metastasis at diagnosis [1]. Metastasis is the most common cause of death in PC patients.

Pancreatic Ductal Adenocarcinoma (PDAC) is an infiltrating epithelial neoplasm with glandular (ductal) differentiation, usually demonstrating luminal or intracellular mucin and without a predominant component of any other histological type.

Currently, there are three recognized precursors of invasive ductal adenocarcinoma: Pancreatic Intraephitelial Neoplasia (PanIN), Intraductal Papillary Mucinous Neoplasm (IPMN) and mucinous cystic neoplasm (MCN) (as it is defined in Bosman: WHO Classification of Tumours of the Digestive System, 4th Edition, 2010)

Pancreatic Intraephitelial Neoplasia (PanIN): microscopic papillary or flat, noninvasive epithelial neoplasms that are usually < 5 mm and confined to pancreatic ducts; composed of columnar to cuboidal cells with variable mucin and divided into three grades according to degree of cytological and architectural atypia which prevalence increases with age.

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Intraductal Papillary Mucinous Neoplasm (IPMN): Intraductal grossly visible (1 cm or more) epithelial neoplasm of mucin producing cells, arising in main pancreatic duct or its branches; neoplastic epithelium is usually papillary; variable mucin secretion, duct dilatation (cyst formation) and dysplasia; classify based on highest degree of cytoarchitectural atypia and invasiveness as: IPMN with low to intermediate grade dysplasia; previously called intraductal papillary mucinous adenoma, IPMN with high grade dysplasia; previously called intraductal papillary mucinous carcinoma, noninvasive and IPMN with associated invasive carcinoma.

Mucinous Cystic Neoplasm (MCN): Benign or potentially low grade malignant cystic epithelial neoplasm composed of cells which contain intracytoplasmic mucin. WHO Classification: with low or intermediate grade dysplasia, with high grade dysplasia or with an associated invasive carcinoma. Almost always in women, mean age 45 years, < 20% associated with invasive carcinoma. Metastases usually restricted to abdominal cavity; metastases to ovary may simulate primary ovarian tumors.

Recent advances in effective screening for early diagnosis of PDAC using novel molecular biomarkers found in different studies have been developed in the last few years. These include different kind of methodologies and different molecular targets but the aim of all these work is to find noninvasive and highly sensitive and specific biomarkers for early detection of Pancreatic Ductal Adenocarcinoma (PDAC).

Among the different methods of Cancer diagnosis, we can find imaging modalities, protein biomarkers [2], mutant DNA templates identified in the circulation (ctDNA) [3], that are derived from cancers and their detection in body fluids (feces, urine, pancreatic juice samples [4] and saliva. DNA promoter hypermethylation [5] and measurement of microRNA as possible noninvasive biomarkers for early detection of PC [6,7].

Proposed biomarkers can be found in different body fluids mainly in blood and also in urine. It is important to focus in High risk population Screening, for identifying early-stage PC and premalignant lesions. Several conditions are associated with a high risk of PC and these can be classified as hereditary or non-hereditary. Examples of common non-hereditary risks are: age (older than 55 years), smoking, obesity, alcohol abuse, diabetes, dietary factors, exposure to toxic substances and chronic pancreatitis [8].

Regarding Hereditary PC, it has been estimated that ten percent of PCs are hereditary. Many of these occur as part of rare medical syndromes such as:

Familial breast cancer gene (BRCA2): BRCA2 was the second familial breast cancer gene identified. It was discovered in 1995 because of a remarkable advance made by the Hopkins team studying PC. The team at Hopkins had discovered a "homozygous deletion" (a missing piece of DNA) in a PC and postulated that this missing piece of DNA is where the BRCA2 gene could be found [9]. Subsequently, Michael Goggins et al. demonstrated that as many as 10% of PC are caused by inherited defects in the BRCA2 gene [10]

Peutz-Jeghers syndrome: This is a very rare hereditary syndrome in which affected family members develop polyps in their small intestines and pigmented spots on their lips and inside of their mouth. Peutz-Jeghers is caused by inherited mutations in the *STK11* gene. Patients with this syndrome have a risk of developing pancreatic cancer that varies between 7% and 36% by the age of 60 [11]

Familial melanoma: The Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome is a rare hereditary syndrome in which affected family members develop skin moles and melanomas (an aggressive form of skin cancer). These patients also have an increased risk of developing PC. FAMMM is caused by inherited mutations in the p16/CDKN2A gene [12,13].

Hereditary colon cancer: The Hereditary Non-Polyposis Colorectal Cancer (HNPCC) syndrome, also known as Lynch syndrome, strikes as many as 1 in 200 individuals and it is characterized by the inherited predisposition to develop colon cancer, endometrial (uterine) cancer, stomach cancer and ovarian cancer. Patients with HNPCC may also have an increased risk of developing PC. Indeed, the DNA finding typical of HNPCC, called microsatellite instability has recently been reported in a small (about 4%) fraction of PCs [14].

Hereditary pancreatitis: This rare disease is characterized by the development of recurrent episodes of severe chronic pancreatitis (inflammation of the pancreas) starting at an early age (often in patients in their teens). The gene responsible for hereditary pancreatitis, called the trypsinogen gene, was discovered by Dr. Whitcomb of the University of Pittsburgh. Clinical gene testing for hereditary pancreatitis is now available [15]. Chronic Pancreatitis is one of the risk factor for PC. Yachida et al., reported that the time from the initiating mutation in the pancreas to development of metastatic PC is almost 21 years [16].

Familial PC: While the above genetic syndromes account for ~20% of familial PC, it is clear that there are other, yet undiscovered familial PC genes. For example, relatives of patients with PC have an increased risk for developing PC themselves, and the National Familial PC Registry now contains over 1500 families in which two or more family members have had PC [17]

As we mentioned before, distant metastasis occurs late during the genetic evolution of PC. Early detection includes an effective screening program, as PC is known to evolve from precursor lesions, which can be currently available diagnostic modalities. A recent multicenter trial, performed by the International Cancer of the Pancreas Screening Consortium (CAPS), CAPS 3 trial, (10) (18) demonstrated that screening of asymptomatic individuals at high risk for PC frequently detects small cystic pancreatic lesions, including curable, noninvasive high-grade neoplasms and that endoscopic ultrasound and magnetic resonance imaging were better at detecting pancreatic lesions than was computed tomography [19]. We also have to take in to account that timely detection of PDAC is masked by several factors: lack of specific clinical symptoms in the early stage of the disease, insufficient sensitivity of current imaging modalities and, despite intensive efforts, lack of accurate body fluid–based biomarkers for early detection. Early-stage PDAC is also difficult to differentiate from Chronic Pancreatitis (CP), a benign inflammatory disease of the pancreas and one of the risk factors for PDAC [20].

Biomarkers in Liquid Biopsies

Biomarkers in Urine

Protein Biomarkers identified in Urine for Early Detection of Pancreatic Adenocarcinoma

A panel of three urine biomarkers that can distinguish patients with early-stage disease from healthy people, which could enable completely noninvasive and inexpensive screening of patients at high risk of developing pancreatic adenocarcinoma has been proposed (2).

Proteins as Biomarkers for Pancreatic Ductal Adenocarcinoma has been studied throughout different approaches to find a strategy for screening purposes of early stages.

Proteomes of 18 urine samples from healthy controls, chronic pancreatitis, and patients with PDAC, (six/group) were assayed using GeLC/MS/MS analysis. Three proteins commonly deregulated in both males and females (LYVE1, REG1A, and TFF1) were selected for further evaluation based in statistical significance and additional literature search for previous knowledge on the potential candidates as researchers now have access to a new analytics features developed to conduct transcriptomic, genomic, mutational as well as integrative analyses using publicly available data [21].

LYVE-1

LYVE-1 is a protein codified by Lymphatic Vessel Endothelial Hyaluronan Receptor 1. This gene encodes a type I integral membrane glycoprotein. The encoded protein acts as a receptor and binds to both soluble and immobilized hyaluronan. This protein may function in lymphatic hyaluronan transport and have a role in tumor metastasis.

REG1A

REG1A also known as islet of Langerhans regenerating protein (REG) is a protein that is encoded by the REG1A gene. This protein is a type I subclass member of the Regenerating protein family. The Reg protein family is a multi-protein family that is involved in the proliferation and differentiation of diverse cell types. For instance, REG3β is an essential soluble factor necessary for PDAC development which is able to stimulate a variety of simultaneous pro-tumoral pathways. Also, REG3β suppresses interactions between epithelial cells and immune cells by activating a signaling cascade mechanism. This mechanism happens to facilitates tumor escape through avoiding immune surveillance, and promotes metastasis. This protein is expressed and released by the far microenvironment, which is situated out of the tumor, at the periphery of the tumor mass, and is part of the healthy peri-tumoral region [22].

REG1A protein that is secreted by the exocrine pancreas, is associated with islet cell regeneration and diabetogenesis and may be involved in pancreatic lithogenesis. Diseases associated with REG1A include Pancreatitis and Acinar Cell Carcinoma.

TFF1

TFF1 belongs to a family of gastrointestinal secretory peptides, which interact with mucins and are expressed at increased levels during reconstitution and repair of mucosal injury. They protect epithelial cells from apoptotic death and increase their motility, but also play similar pivotal roles in cancer cells, and are thus involved in the development and progression of various cancer types. In PDAC, TFF1 has been reported in both sporadic and familial PanINs, and it has been associated with early stages (I and II) of the disease and disease without lymph node involvement, i.e., stage IIA. This expression mimics the pattern seen in our urine samples and confirms the biologic importance of TFF1 as an early diagnostic biomarker.

Tomasz P. Radon et al, (2) have recently reported high levels of the LYVE1, REG1A, and TFF1 in urine samples from patients with PDAC. The presence of as full-size proteins in urine specimens was confirmed by Western blot and subsequently validated with ELISA assays.

When levels of LYVE1 and REG1A were measured after the surgery, these biomarkers were significantly decreased. This finding also applies to TFF1 regarding a smaller group of patients. This fact suggests that the substantial decrease of concentration of these biomarkers in urine samples after surgery would be due to the loss of tumor mass after the surgery (2).

CA19.9

Cancer antigen 19-9 is used to help differentiate between cancer of the pancreas and other conditions, as well as to monitor treatment response and recurrence. This antigen is tested in blood samples and is used primarily in the management of PC. When analyzing CA19.9 tumor marker levels were higher in PDACs stage I–II compared with pancreatic neuroendocrine tumors (NETs) and duodenal cancer samples. When the protein Biomarkers used for detection of early stages of PA are combined with CA19.9, accuracy may be increased, which may help to understand a recent finding that serum CA19.9 is upregulated up to 2 years before PDAC diagnosis [23].

Then a protein Biomarker panel (LYVE1, REG1A, and TFF1) has been proposed and a diagnostic test based on urine specimens has been developed taking into account that urine is less complex than blood and the possibility of obtaining sufficient sample volumes as much times as needed in a noninvasive fashion.

It can be pointed out that other of the advantages of urine as a sampling method is that this body fluid, constitutes an ultra-filtrate of blood, in this way higher concentration of biomarkers can be expected. Nevertheless, gender specific differences in protein composition of bio fluids were found (24), this should be also analyzed in further studies on the subject.

Biomarkers in blood samples

Liquid Biopsy: Circulating tumor DNA and protein biomarker-combined for early detection of PCs

ct DNA is DNA circulating that can be found in different body fluids. ctDNA coming from dying cancer cells is being targeted. These samples of ctDNA can be obtained from body fluids as urine, blood, stool, saliva, bile cerebrospinal fluid and pancreatic juice (25, 26, 27, 28) by means of "liquid biopsies". Factors released by the tumor far microenvironment are decisive for pancreatic adenocarcinoma development and progression.

KRAs oncogene, (Kirsten ras oncogene homolog gene), produces a small G protein downstream of Epidermal Growth Factor Receptor (EGFR). A single amino acid substitution causes an activating mutation. Somatic (non-germline) mutations are frequently detected in endoderm derived tumors, including colon, lung and pancreas, perhaps because activated Kras promotes the expansion of an endodermal stem / progenitor cell and blocks its differentiation (29,30). The KRAS oncogene, is a potent driver of tumor initiation and maintenance. Inactivating mutations in tumor suppressor genes such as CDKN2A/p16, TP53, and SMAD4 cooperate with KRAS mutations to cause aggressive PDAC tumor growth [31]. Combining ctDNA and protein markers increases sensitivity because a large proportion of patients are detected by only one marker [32,33].

An advantage of using mutant DNA in the circulation as a biomarker is its exquisite specificity. Every cell within a cancer has a core set of somatic mutations in driver genes that are responsable for their clonal growth (34, 35). In contrast, normal cells do not clonally expand during adulthood, and the fraction of normal cells that have any specific somatic mutation is extremely low.

Most studies of Circulating Tumor DNA (ctDNA) have focused on following patients with cancer rather than on evaluating its use in screening settings. Available data indicate that ctDNA is elevated in >85% of patients with advanced forms of many cancer types. However, it has been shown that a considerably smaller fraction of patients with earlier stages of cancer have detectable levels of ctDNA in their plasma [36,37].

ctDNA and protein biomarkers can be combined to increase the sensitivity and preserve high specificity under certain conditions. Joshua D. Cohen, et al. designed a PCR-based assay that could simultaneously assess the two codons (codons 12 and 61) of the KRAS gene that are most frequently mutated in PDAC as well as surrounding codons (3). Then it is therefore important to determine whether the KRAS mutations identified in these patients' plasma samples were also present in their primary carcinomas.

When Primary carcinomas were obtained by surgery from patients, KRAS mutations in their plasma were detected in 75.75 % of them. In all cases, the mutation found in the plasma was identical to that found in the primary carcinoma, this finding provides another significant of specificity [38].

Simultaneous Assessment of CA19-9 and KRAS Mutations in Plasma were assayed in order to determine whether a combination of the KRAS ctDNA test with CA19-9, the best-known PDAC biomarker [29], would result in improved sensitivity compared with the KRAS ctDNA test alone. It is important to point out that in Lennon AM and Goggins M study [29], the samples were obtained only from patients with resectable PCs, patients with advanced disease were excluded.

A recent study of Joshua Cohen et al. (3) has shown that CA19-9 can be elevated in patients with PC, 2 years before diagnosis. However, CA19-9 elevations have also been observed, in nonmalignant conditions, and 5% of the population cannot produce the CA19-9 antigen due to germline genetic variation, limiting its use for screening purposes. It is important to highlight that whether these two biomarkers KRAS mutations and a positive CA19-9 score were independent indicators of the presence of disease. However, if the threshold for scoring CA19-9 result as positive was sufficiently high it might be useful as a screening biomarker. Joshua Cohen et al, (3) used a threshold of 100 U/mL based on prior data that this level is not

found among healthy individuals who do not have a clinical history of pancreaticobiliary disease.

Regarding this assessment, Joshua Cohen et al, found the combined sensitivity of these analysis was significantly higher than the sensitivity of either alone. Importantly, the two assays: one identifying ctDNA carrying specific Kras mutations associated with PC and the other one combining simultaneous assessment of CA19-9 and KRAS Mutations in Plasma, could be combined without substantially increasing the false-positive rate because each was extremely specific at the thresholds used.

Other cell free DNA selected genes Biomarker

DNA Promoter or (O6-alkylguanine DNA alkyltransferase) also known as MGMT is a protein that in humans is crucial for genome stability. It repairs the naturally occurring mutagenic DNA lesion O6-methylguanine back to guanine and prevents mismatch and errors during DNA replication and transcription. Accordingly, loss of MGMT increases the carcinogenic risk in mice after exposure to alkylating agents [39].

DNA promoter hypermethylation is a mechanism of early carcinogenesis, which can cause inactivation of tumour suppressor genes. The aim of this study was to examine promoter hypermethylation in a panel of selected genes from cell-free DNA, as a diagnostic marker for pancreatic adenocarcinoma. In patients with pancreatic adenocarcinoma, chronic pancreatitis, acute pancreatitis and patients screened, but negative for pancreatic adenocarcinoma, the difference in mean number of methylated genes in the cancer group vs the total control group was highly significant (5).

Cell-free DNA promoter hypermethylation has the potential to be a diagnostic marker for pancreatic adenocarcinoma and differentiate between malignant and benign pancreatic disease [40,41]. External validation is, however, required before the test can be applied in the clinic.

Trefoil factor family 2 (TFF2) present in Pancreatic Duct Glands, as possible protein biomarker to early detection of Intraductal Papillary Mucinous Neoplasms

Pancreatic Duct Glands (PDGs) are small glands that have molecular features known to mark stem cell niches, it is proposed the role of PDG as a progenitor niche [42]. These glands are located over the main pancreatic ducts that function as progenitor niche for the ductal epithelium and they express gastric mucins. Junpei Yamaguchi et al. investigated, whether PDGs are a precursor compartment for Intraductal Papillary Mucinous Neoplasms (IPMN) a type of neoplasm about little is known and the role of trefoil factor family 2 (TFF2), a protein expressed by PDGs and the gastric mucosa that are involved in epithelial repair and tumor suppression [43].

A variety of genetically engineered mouse models of PC have been developed over the last decade. PDAC is distinguished by four genes that are altered in a very high fraction of patients: the K-ras proto-oncogene is mutationally activated in >90% of cases. Given the frequency of K-ras mutations in PDAC and their presence in the earliest lesions, activation of oncogenic K-ras signaling has been proposed to be the primary initiating event in pancreatic carcinogenesis [44]. The KC mice model is a genetically model that express the KRasG12D oncogene in all pancreatic lineages from early embryonic development and incorporates only an activating point mutation in Kras that is conditionally expressed in the pancreas. Expression of K-rasG12D specifically to the pancreas was targeted in this model. Although the mice were born with normal pancreatic histology and architecture, an increased pancreatic mass at birth was the only difference between double transgenic mice and controls. However, by 8 weeks of age, the mice began to develop early lesions that slowly increased in both number and grade over the next 2 years. A subset of these mice developed pancreatic ductal adenocarcinoma, with a median overall survival of 14 months [44]. It is important to highlight that the K-rasLSL.G12D/+; PdxCre model (now referred to as the KC model) developed a significant background tumor spectrum.

This model provided proof that K-ras mutations are sufficient to initiate PC formation in mice [45].

When KC mice, with a genotype that made them able to express K-ras mutations and can release protein Tff2 (KC/Tff2+/+) and other KC mice with genotype that made them able to express K-ras mutations and cannot release protein Tff2 (KC/Tff2−/−), mice developed prominent papillary structures in the duct epithelium with cystic metaplasia of the PDG, which resembled human IPMN. Expression of TFF2 reduced proliferation of PDAC cells 3-fold; this effect required upregulation and activation of SMAD4. SMAD4 is a gene that codifies a protein that acts as a tumor suppressor and inhibits epithelial cell proliferation. It may also have an inhibitory effect on tumors by reducing angiogenesis and increasing blood vessel hyperpermeability. Loss of TFF2 accelerates tumorization of KC mice. Junipei Yamaguchi et al., found expression of TFF2 to be down regulated in human PDAC by hypermethylation of its promoter.

According to these findings we can think of TFF2 expression as a possible early detection biomarker for Intraductal Papillary Mucinous Neoplasms and even though PDAC.

Measurement of microRNA as possible noninvasive biomarker for diagnosis of in early detection of PC.

The miRNAs are non-coding RNAs that regulates gene expression and have been recognized as deregulated in oncogenesis. miRNAs are very stable in blood because they are usually bound to Argonaute, a protein that protects them from RNase degradation. Several authors reported that miRNAs are deregulated in pancreatic diseases being able to discriminate PC from pancreatitis, pancreatic precursor lesions and healthy individuals and precursor lesions such as Intraductal Papillary Mucinous Neoplasms (IPMN), with malignant potential being present in several types of samples [46,47,48].

The miRNAs have been profiled as one of the most popular non-invasive biomarker in early detection of PC and numerous of these molecules are shown to be deregulated while and after PC development. Validations in larger cohorts and consensus regarding definitive panels are need before clinical applications would be real for patients.

Several miRNAs were identified that exhibited abundance variations in both tissue and blood samples. The results could have an immediate diagnostic value for the evaluation of tumor reoccurrence in patients, who have undergone curative surgical resection, and for people with a familial risk of pancreatic cancer. Several miRNAs were found to be associated with tumor-relevant processes. Similar to mRNA profiling, miRNA signatures exhibited distinctive expression variations in pancreatic tumor samples, chronic pancreatitis tissue and normal pancreas. The transcripts miR-21, miR-155, miR-203, miR-210 and miR-222 were described as potential predictors of survival. However, the tissue-based miRNA studies also suffer from the fact that invasive action is required to acquire material for analysis, and as such, tissue-based miRNA profiling does not offer significant progress compared to messenger RNA profiling but for the superior stability of miRNA.

Markers that occur in peripheral blood or other body fluids would be best for detection. For various tumor entities, extracellular nucleic acids have been found in serum. In part, they have their origin in circulating tumor cells. Moreover, the actual tumor cells themselves could be isolated from blood and used as a means for diagnosis and prognosis.
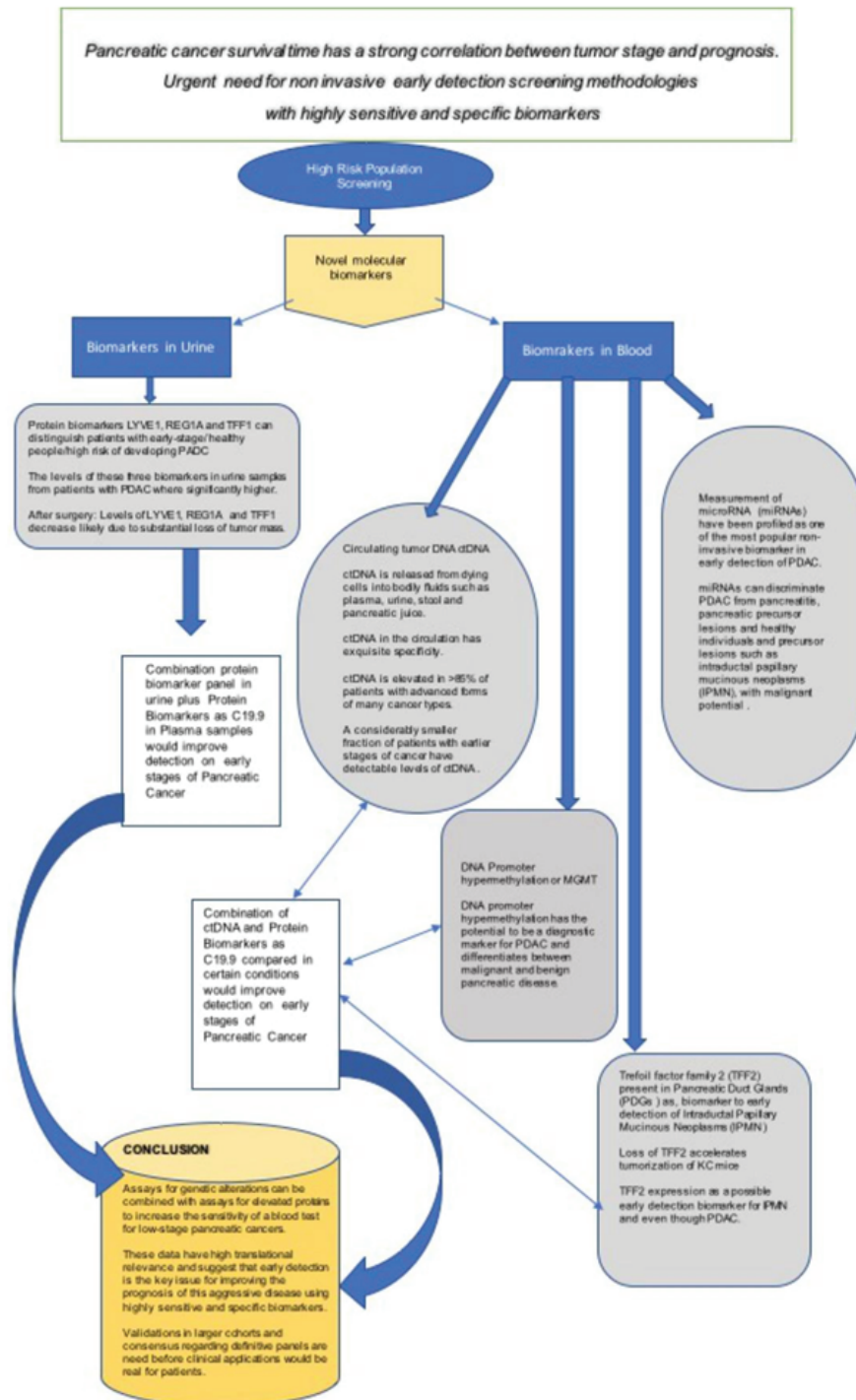
Conclusion

Pancreatic cancer survival time has a strong correlation between tumor stage and prognosis. Performing High Risk population screening, by means of Biomarkers in liquid biopsies, would lead us to remarkable achievements in the subject. As shown in Figure 1.

In this article, we have reviewed biomarkers in urine and biomarkers in blood.

Three urine protein biomarkers, noninvasive inexpensive that can distinguish patients with early-stage/ healthy people/high risk of developing PDAC: LYVE1, REG1A, and TFF1. The levels of these

Figure 1

Figure 1

three biomarkers in urine samples from patients with PDAC, where significantly higher. In patients for whom samples were collected before and after surgery, levels of LYVE1 and REG1A and also TFF1 decrease likely due to substantial loss of tumor mass after surgery. Combination of protein biomarker panel in urine, plus protein biomarkers as C19.9 in plasma samples would improve detection on early stages of Pancreatic Cancer.

Nevertheless, biomarkers in blood are also liquid biopsies as circulating tumor, (ctDNA), released by dying cancer cells than can escape into body fluids such as urine, blood, stool and pancreatic juice. In this review, we pointed out that mutant DNA circulating in Blood is an exquisite marker in terms of specificity as it is elevated in › 85% of patients with advanced forms of many cancer types. Combination of ctDNA and Protein Biomarkers as C19.9, compared in certain conditions would improve detection on early stages of

Pancreatic Cancer.

Another two useful biomarkers in blood would be measurement of microRNA (miRNAs) and DNA promoter hypermethylation.

MicroRNA have been profiled as one of the most popular non-invasive biomarker in early detection of PC. These molecules are non-coding RNAs very stable in blood, that regulates gene expression and have been recognized as deregulated in oncogenesis. In pancreatic diseases, they can discriminate PC from pancreatitis, healthy individuals and pancreatic precursor lesions such as Intraductal Papillary Mucinous Neoplasms (IPMN), with malignant potential.

DNA promoter hypermethylation is a mechanism of early carcinogenesis. DNA Promoter, also known as MGMT, repairs the naturally occurring mutagenic DNA lesions and prevents mismatch lesions and errors during DNA replication and transcription. Cell-free DNA promoter hypermethylation has the potential to be a diagnostic

marker for pancreatic adenocarcinoma and differentiate between malignant and benign pancreatic disease.

On the other hand, possible use of protein factors as Trefoil Factor Family2 (TFF2) present in Pancreatic Duct Glands (PDGs) as biomarker to early detection of Intraductal Papillary Mucinous Neoplasms (IPMN). The role of Trefoil Factor Family 2 (TFF2), a protein expressed by PDGs and the gastric mucosa that is to be involved in epithelial repair and tumor suppression. KC mice model as was described above, provided proof that K-ras mutations are sufficient to initiate pancreatic cancer formation in mice. Loss of TFF2 accelerates tumorization of KC mice and expression of TFF2 to be down regulated in human PDAC by hypermethylation of its promoter. Then we can think of TFF2 expression as a possible early detection biomarker for Intraductal Papillary Mucinous Neoplasms and even though PDAC.

Based on data provided in the items commented above we can conclude that assays for genetic alterations can be combined with assays for elevated proteins to increase the sensitivity of a blood test for low-stage pancreatic cancer.

Larger scale and rigorous validation is required before their application in the clinic. In addition, more effective and specific biomarkers of PDAC and PDAC pathogenesis at the genetic and molecular level, as well as novel therapeutic opportunities to treat this highly aggressive disease are urgently needed. Fortunately, as it was mentioned above, the increased number and importance of Translational studies in the subject, would lead us to achieve this remarkable tool in a near future. A novel method combining Protein biomarkers and in urine and the challenge of identifying, (ctDNA) mutant DNA templates in urine could lead us to a less complex, method that provides a stable matrix for analysis, and can be repeatedly and noninvasively sampled in sufficient volumes.

A useful biomarker with applicability for early diagnosis should be minimally or non-invasive and have high sensitivity, high specificity and capacity to discriminate low-grade dysplasia from high-grade dysplasia and cancer.

Minimally-invasive or non-invasive biomarkers are required to encourage use for clinicians and compliance in patients. Only non-invasive tests are practical enough and will be able to maximize access. To obtain these properties, biomarkers in blood, urine, saliva, feces or pancreatic juice samples must be more deeply investigated.

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