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Review

Biochemical diagnostics of pancreatic cancer - Present and future

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ABSTRACT

Pancreatic cancer is one of the deadliest cancers having an exceptionally high mortality rate. Despite a relatively low incidence (10th among cancers), it is the fourth leading cause of cancer-related deaths in most developed countries. Improving early diagnosis of pancreatic cancer and strengthening the standardised comprehensive treatment remain the main focus of pancreatic cancer research. Tumor markers are usually tumor-associated proteins of clinical relevance in these patients. Although tumor markers carbohydrate antigen (CA 19–9) and carcino-embryonic antigen (CEA) are commonly used, neither demonstrate high diagnostic accuracy. Recently, hematopoietic growth factors (HGFs) and various enzymes have been reported as potential biomarkers for pancreatic cancer. These include macrophage-colony stimulating factor (M-CSF) and granulocyte-colony stimulating factor (G-CSF), interleukin-3 (IL-3), macrophage inhibitory cytokine (MIC-1) and various enzymes (alcohol dehydrogenase, aldehyde dehydrogenase, lysosomal exoglycosidases). With the development of molecular technology, detecting K-ras mutation in serum via polymerase chain reaction (PCR) is becoming more common and efficient. Because K-ras mutation rates are high in many cancers, some regard it as a potential tumor marker. Others have shown the value of serum miRNAs in detection of pancreatic cancer. Unfortunately, there are currently no effective methods of sufficient diagnostic accuracy to detect early-stage surgically resectable pancreatic cancer. In this article we highlight these biomarkers and summarise recent developments in the diagnosis and treatment of pancreatic cancer.

1. Introduction

Pancreatic cancer (PC) is an aggressive and devastating disease characterised by invasiveness, rapid progression and profound resistance to treatment. Despite the low incidence of PC relative to other cancers, it is considered one of the most lethal in most developed countries. For example, pancreatic cancer accounts for about 3% of all cancers and about 7% of cancer deaths in the United States of America, with a median survival rate of < 6 months [1]. Unfortunately, PC has nonspecific symptoms in the early stages and, as such, it is usually diagnosed at an advanced stage, when curative treatment is generally ineffective. Blood tests are commonly used to assess liver function for patients with jaundice to determine cause of pancreatic cancer. Prognosis is strongly correlated with pathologic stage at the time of diagnosis. Commonly used noninvasive imaging modalities, including transabdominal ultrasound and computed tomography (CT), have a relatively low diagnostic yield for early pancreatic cancer due to small size. CT is, however, the imaging modality of choice for staging PC [2,3]. As such, it is crucial to find specific markers which detect any neoplastic transformation. Such markers would have a significant impact on early detection and diagnosis thus leading to improved management and treatment outcome [4]. Tumor markers are substances which can be detected at concentrations exceeding normal physiologic levels in blood, urine or other body fluids. They are synthesised and excreted by the tumor tissue itself. An ideal marker should possess very high specificity (i.e. it is not detectable in healthy subjects) and very high sensitivity (i.e. it is detectable very early when few cancer cells are present). It should have high predictive value and should correlate with tumor stage. Unfortunately, 100% sensitivity and 100% specificity have not yet been fulfilled by any known tumor marker [5,6]. Recently,

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AUC ROC, area under the ROC curve; CA19-9, carbohydrate antigen 19-9; CA 242, carbohydrate antigen 242; CA 50, carbohydrate antigen 50; CEA, carcinoembryonic antigen; CEER™, Collaborative Enzyme Enhanced Reactive-immunoassay; CEMIP, cell migration-inducing protein; CSF, colony stimulating factors; CT, computed tomography; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage-colony stimulating factor; HEX, *N*-acetyl-β-D-hexosaminidase; HEX A, isoenzyme A of *N*-acetyl-β-D-hexosaminidase; HGFs, hematopoietic growth factors; IL-3, imterleukin 3; M-CSF, macrophage-colony stimulating factor; MIC-1, macrophage inhibitory cytokine; miRNA, micro RNA; OS, overall survival; PC, pancreatic cancer; PCR, polymerase chain reaction; SCF, stem cell factor; TGF-β, transforming growth factor-beta

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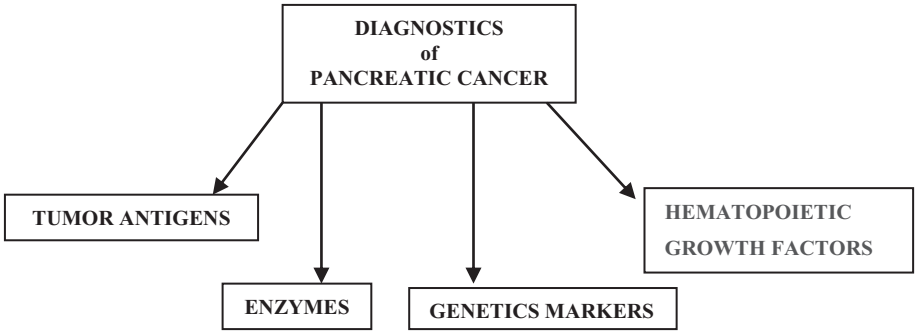

Fig. 1. Division of pancreatic cancer markers.

panels of biochemical markers derived from blood, pancreatic juice, saliva, stool and pancreatic tissue have been evaluated as promising markers for PC, but most of them have low sensitivity or specificity. For example, *K-ras* mutation detection in pancreatic juice is associated with poor sensitivity (between 38 and 62%) and is of poor diagnostic value because these mutations can be found in chronic pancreatitis [7].

Tumor markers can be categorized into several groups: tumor antigens, hematopoietic cytokines, enzymes, and genetic markers (Fig. 1, Table 1).

2. Tumor antigens

Currently, the only available biomarker approved for use in pancreatic cancer is carbohydrate antigen CA 19-9, which was first discovered in 1979. It is a glycoprotein characterised by high molecular weight which may be released to the blood. CA 19–9 is mainly synthesised by the pancreatic duct, as well as epithelial cells of the gastrointestinal tract and biliary system. Moreover, CA 19-9 is produced by pancreatobiliary and gastric tumors. CA 19-9 is widely used in screening, diagnosis, staging, early identification of recurrence, as well as in predicting treatment response [8]. It is highly recommended for monitoring PC treatment response. Unfortunately, the use of CA 19-9 has several limitations. Specificity is questionable and its expression is not high in early stage PC. Moreover, it is not universally expressed in PC. This marker, typically used for colorectal and gastric cancer diagnosis, is increased in malignant biliary tract and benign obstructive jaundice [9]. CA 19-9 values of up to 37 U/mL are considered normal antigen in serum. Using 80% sensitivity and > 80% specificity as criteria, Kim et al. showed that the preoperative CA 19-9 might predict early distant metastasis in PC [10]. However, the role of CA 19–9 kinetics as a prognostic tool for patients treated with palliative first-line chemotherapy remains contradictory. Although several studies reported a significant improvement in overall survival (OS) with decreasing CA 19-9 (20–89%), a recent large-scale study conducted by Hess et al. did not confirm these findings [11–13].

Several studies have investigated the role of cell migration-inducing protein (CEMIP) in PC. CEMIP appears associated with early detection, cancer cell migration, invasion and poorer prognosis. Combined CA 19-9 and CEMIP showed markedly improved AUC ROC vs CA 19-9 alone in PC diagnosis (0.94 vs 0.89; $P < .0001$). CEMIP revealed a diagnostic yield of 86.1% (68/79) in CA 19–9 negative PC [14,15]. As such, CEMIP may provide a complementary marker in PC with normal CA19-9 [16]. Although CA 19-9 is known as a PC biomarker, it is not commonly used for general screening due to low sensitivity and specificity. Falsely negative results have been reported for those with Lewis blood type A-B and falsely positive results have been reported in patients with obstructive jaundice. According to the National Academy of Clinical Biochemistry, CA 19-9 alone does not permit identification of PC recurrence without confirmation by clinical symptoms and/or biopsy [9].

Carcinoembryonic antigen (CEA), first detected in 1965, has been

Table 1
Diagnostic criteria for markers of pancreatic cancer.

Group/markers	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Area under ROC Curve
Tumor antigens			
Carbohydrate antigen CA 19-9 [21]	77	100	0.9148
Carcinoembryonic antigen CEA [21]	37	100	0.9091
Carbohydrate antigen CA 50 [22]	96	48	0.7380
Carbohydrate antigen CA 242 [23]	75	85,5	0.8354
Hematopoietic growth factors (HGFs)			
Stem cell factor (SCF) [21]	98	17	0.9018
Granulocyte-colony stimulating factor (G-CSF) [21]	19	96	0.5133
Macrophage-colony stimulating factor (M-CSF) [21]	61	96	0.8481
Granulocyte-macrophage-colony stimulating factor (GM-CSF) [21]	69	70	0.7703
Macrophage inhibitory cytokine (MIC-1) [30]	94	46	0.9170
Enzymes			
Alcohol dehydrogenase (ADH) [35]	57	65	0.5784
Isoenzyme class III of ADH [35]	70	76	0.6457
N-acetyl-β-D-hexosaminidase (HEX) in serum [39]	93	100	0.9778
N-acetyl-β-D-hexosaminidase (HEX) in urine [39]	87	86	0.9000
Cathepsin D [40]	78	83	no data
Genetic markers			
K-ras mutation [47]	81.8	81.5	0.85
miR-642b-3p, [51]	82	56	0.79
miR-885-5p, [51]	82	73	0.84
miR-22-3p [51]	82	82	0.86
Combination of 3 miRNAs [51]	91	91	0.97

commonly employed as a biomarker in colorectal cancer [17]. Although increased CEA has been reported in > 60% of patients with pancreatic ductal adenocarcinoma, few studies have investigated its clinical significance in PC [18–20]. CEA is a marker that might help identify advanced PC in some patients, but it is not used as often as CA 19–9. Diagnostic sensitivity of CEA is lower than CA 19-9 [21]. CA19–9 or CEA can be monitored in patients with known PC to determine treatment efficacy and can identify recurrence.

Potential carbohydrate markers of PC include carbohydrate antigen 50 (CA 50) and carbohydrate antigen 242 (CA 242). The former is not organ specific and can be increased in the serum in a variety of malignancies especially gastrointestinal cancer. Although CA 50 is similar to CA 19-9 with little diagnostic value, it is very useful in monitoring PC. Diagnostically, CA 50 has high sensitivity (96%), but low specificity (48%) [22]. CA 242 is a tumor marker whose structure remains unresolved. However, there is evidence that CA 242 has a sialylated carbohydrate type I chain different from CA 19-9 and CA 50. Ozkan et al. compared the diagnostic value of CA 242 and CA 19-9 with CEA in PC and found a positive correlation between CA 242 and CA 19-9. In PC, CA 242, CA 19-9 and CEA demonstrated sensitivity of 75, 80 and 40% and specificity 85.5, 67.5 and 73%, respectively [23].

Unfortunately, none of these tumor markers is sufficiently accurate to diagnose PC. Levels are not high for all patients with PC and others have high levels for other reasons.

3. Hematopoietic growth factors

PC cells produce hematopoietic growth factors (HGFs). Stem cell factor (SCF), granulocyte-colony stimulating factor (G-CSF), macrophage-colony stimulating factor (M-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF) are all members of glycoprotein cytokines called colony stimulating factors (CSFs) or HGFs. Cytokines may stimulate carcinogenesis and growth of cancer cells in an autocrine manner and several cell lines of malignant tumors secrete large amounts of HGF and express their receptors [24]. Cytokines can also modulate several processes involved in tumor progression and metastasis, ie, angiogenesis and production of metalloproteinases [25]. Pei et al. suggested that the enhanced production of extracellular matrix-degrading proteinases by cancer cells in response to treatment with hematopoietic cytokines may represent a biochemical mechanism that promotes invasive behaviour [26]. Moreover, Esposito et al. showed that SCF, also known as the ligand c-kin receptor, may have a growth-regulating role in the normal pancreas and is altered during malignant transformation [27]. Increased serum hematopoietic cytokines have been reported in PC [28,29]. Groblewska et al. found that serum M-CSF and G-CSF were significantly increased in PC vs controls. Increased M-CSF and G-CSF in more advanced PC might result from cytokine production by cancer cells. Diagnostic sensitivity and specificity of M-CSF were 37% and 95%, respectively. Diagnostically, sensitivity, specificity and the AUC ROC were substantially higher for M-CSF vs G-CSF. Unfortunately, M-CSF had very low sensitivity thus disqualifying it as a tumor marker. Groblewska et al. demonstrated the usefulness of M-CSF as a tumor marker for PC, especially in combination with CA 19–9. The diagnostic sensitivity of this combination (84%) was higher than CA 19–9 with G-CSF or CA 19–9 with CEA [29]. Other studies showed increased proinflammatory cytokines, such as interleukin-3 (IL-3) or granulocyte-macrophage-colony stimulating factor (GM-CSF) and SCF. In serum, median GM-CSF and IL-3 in PC was significantly higher in PC, but SCF was significantly lower vs controls [29]. Although several studies suggested that HGFs were candidates for pancreatic tumor markers, further investigation is clearly required.

Mohamed et al. showed that circulating macrophage inhibitory cytokine (MIC-1) in PC serum was significantly higher than healthy controls [30]. MIC-1, a distant member of the transforming growth factor-beta (TGF-β) family of cytokines, was originally identified as a gene expressed in macrophage activation. Several studies have shown that MIC-1 was over-expressed in PC vs other pancreatobiliary diseases or healthy subjects [31,32]. As compared to CA 19–9, MIC demonstrated higher sensitivity but lower specificity and this difference was related to early vs late stage detection. Diagnostically, MIC-1 demonstrated 94% sensitivity and 46% specificity with 78.3% positive and 78.6% negative predictive value [30].

4. Enzymes

In recent years a number of studies have focused on the application of enzymes, including alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), lysosomal exoglycosidases and cathepsin D, as PC markers. Jelski et al. demonstrated that ADH was significantly higher in PC tissues whereas ALDH was unchanged [33]. Moreover, results indicated that only class III ADH activity (the most important isoenzyme in the pancreas) was significantly higher in PC vs healthy tissue. Increased cancer cell enzyme activity is reflected in the serum. For example, serum total ADH activity changed in the course of PC and was was positively correlated with increased class III ADH isoenzyme [34]. Furthermore, the serum activity of ADH total and ADH III was higher in advanced-stage PC. Diagnostically, ADH III demonstrated 70% sensitivity, 76% specificity with 79% positive and 71% negative predictive value. The area under the ROC curve for ADH III was 0.64. These results suggest a potential role of ADH (especially ADH III) as a marker for PC, but further investigation and confirmation by prospective studies are necessary [35]. Determination of alcohol dehydrogenase activity can be conducted in most laboratories.

Normal stem cells were shown to contain increased ALDH activity vs their more differentiated progeny, ie, the transit amplifying cells. In support, Rasheed et al. demonstrated that the identification of PC stem-like cells was possible through a marker-dependent cell and that the cells that were proven to possess tumor-initiating properties with increased intracellular aldehyde dehydrogenase in addition to the expression of cell surface markers CD 133, CD 24 and CD 44 [36].

Cancer cells are capable of producing a variety of hydrolytic enzymes, including lysosomal exoglycosidases. The source of exoglycosidases can be white blood cells, such as macrophages, neutrophils and lymphocytes that accumulate around the tumor as a result of inflammation and the damaged pancreatic tissue itself [37]. Szajda et al. noted a significant increase of *N*-acetyl-β-D-hexosaminidase (HEX) and its isoenzyme A (HEX A) in the serum and urine of patients with PC [38]. The exoglycosidase with the highest sensitivity and specificity as a PC marker was HEX in both serum and urine. Diagnostically, HEX demonstrated 93% sensitivity and 100% specificity in serum and 87% sensitivity and 86% specificity in urine. Szajda et al. suggested using lysosomal exoglycosidase profiles in the differential diagnosis of PC [39]. In addition, cathepsin D activity in serum and urine may be useful tool in diagnosis of PC. Although cathepsin D demonstrated 78% sensitivity and 83% specificity in serum, additional studies are needed to validate this potential biomarker [40].

5. Genetic markers

Biomarkers described above are currently available and used in the diagnosis of PC. However, it is likely that genetic markers will continue to evolve and represent more robust tools in detecting neoplastic changes in this organ.

The *Ras* family represents the most common oncogene mediating signal transduction pathways involving inflammation and cell proliferation. K-ras, a common human oncogene, binds guanine nucleotides involved in growth factor signal transduction whereas pathologic mutation leads to cell proliferation [41]. Some consider K-ras mutation as an important and early event in tumorigenesis [42,43]. Reports have shown that the K-ras mutation occurs in 47–100% of pancreatic ductal adenocarcinomas, most commonly at codon 12 [44,45]. In serum *K-ras* mutations may be detected via polymerase chain reaction (PCR) [46],

and as such may be useful for diagnosis of PC. Gu et al. reported that CA 19–9 was highly sensitive and K-ras highly specific method in diagnosing PC due to their different roles in pancreatic carcinoma [47]. However, some hold an opinion that the relationship between the K-ras mutation and PC is not strong [48]. A novel magnetic nanoprobe system was able to detect K-ras mutations in fecal samples from patients with PC at a higher rate than patients with benign pancreatic disease and healthy controls. The diagnostic sensitivity for this method was 81.8%, specificity was 81.5% and positive and negative predictive values were 87.8% and 73.3% respectively. The K-ras mutation detection method had greater sensitivity and specificity than CA19–9 [49].

Recently, microRNAs (miRNAs) have been reported as potential biomarkers for various types of cancers. [50–53]. It should be noted that each of the previous studies suggested different circulating miRNA markers for PC. For example, using plasma samples from fifty cancer patients and ten healthy subjects, as well as chemo-resistant pancreatic cell lines, Ali et al. suggested that serum miR-21and other miRNAs could predict the PC aggressiveness [50]. Similarly, Ganepola et al. examined plasma from patients with and without PC and concluded that three miRNAs, ie, miR-642b, miR-885-5p, and miR-22, were > 90% sensitive and specific as diagnostic tools [51]. Kojima et al. suggested that assessment of eight combined miRNA markers (miR-6075, miR-4294, miR-6880-5p, miR-6799-5p, miR-125a-3p, miR-4530, miR-6836-3p, and miR-4476) was clinically valuable to identify pancreatobiliary cancers in patients who could potentially benefit surgically [54]. Such a test will provide additional information to those who can be at risk and, if necessary, prompt the use more costly and sometimes invasive imaging studies. Although the biologic roles of miRNAs are under discussion, these unique markers could potentially lead to early detection and improved survival.

Current genomics-based technologies are unable to reflect the quantitative dynamic signaling changes within the tumor. As such, “functional” diagnostics based on proteomics may serve as a useful complementary tool by providing the most direct link to cancer cell phenotype [55]. As most targeted agents are signaling modulators, a proteomics-based diagnostic will be extremely helpful in assessing treatment response, identifying potential resistance mechanisms and guiding further treatment decision, all of which are impossible using archived tumor tissue. Lim et al. reported the use of a multiplex proteomic-based assay, Collaborative Enzyme Enhanced Reactive-immunoassay (CEER™). The CEER™ platform allows simultaneous detection of the abundance and activation status of multiple key signaling molecules uniquely deregulated in biopsied PC but not normal tissue. The authors demonstrated that this technique is clinically feasible, highly sensitive, specific and reliable. In the current era of precision oncology, this technique could provide important proteomic information within the tumor critical in assessing the treatment effect and providing secondary signaling changes that could identify resistance mechanisms [56].

6. Summary

Early diagnosis of PC is difficult due to negligible clinical symptoms at early disease stage. Prognosis is generally poor due to its rapid infiltration of surrounding tissues and early formation of metastases. Imaging methods and laboratory tests all play an important role in the diagnosis of PC. Cancer cells often secrete specific glycans, which may be useful as diagnostic tools. These glycans, appearing in free or covalent complexes with proteins and lipid are membrane-associated or secreted. The most widely used and best-recognised carbohydrate marker of PC is CA 19-9. Unfortunately, no currently available tumor marker has sufficient diagnostic sensitivity and specificity. As such, development of novel diagnostic markers is needed. It is likely that various combinations of existing and developing markers will provide additional insight as diagnostic, prognostic and therapeutic tools. Molecular approaches such as analysis and characterization of miRNAs will also likely play an important role in future approaches to PC.

Declaration of Competing Interest

None.

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