Serum Tumor Markers as Screening Tools in Pancreatic Adenocarcinoma

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Abstract

Pancreatic adenocarcinoma is not the most frequent cancer, but it is one of the deadliest cancers. The 5-year survival rate is 9% only. It is usually diagnosed at a later stage when surgical resection is not possible and metastases have already spread to other parts of the body. Developing early screening tools can help in diagnosing pancreatic cancer at initial and earlier stages when surgical resection is still a possibility leading to more years of survival and a potential cure. Different studies show the importance of identification of pancreatic carcinoma before large growth in tumor size. Recurrence after surgery is late if serum tumor marker level is comparatively less, tumor size is small and it is localized with no lymph node or vascular invasion and so according to different studies, early detection of tumor lead to more median survival time. There are many pancreatic carcinoma serum tumor markers, but most of them do not improve upon CA19-9 which is considered a gold standard pancreatic carcinoma serum marker although CA 19-9 also does not have perfect sensitivity and specificity. Because of less than 100% sensitivity and specificity, screening tests, using serum markers are mostly not recommended for the general population, although they are recommended for high-risk populations. Here we present a brief review about Ca 19-9 and some other pancreatic adenocarcinoma serum tumor markers including THBS2, MIC-1, sTRA, CEACAM1, CA 494, Span-1 and PAM4 which show some improvement over CA19-9. CA 19-9 is considered the standard pancreatic adenocarcinoma serum tumor marker but there are other pancreatic adenocarcinoma serum tumor markers with comparatively better sensitivity and specificity. THBS2 has comparatively less sensitivity but better specificity compared to CA 19-9. PAM4 has comparatively less or equal sensitivity but better specificity compared to CA 19-9. CEACAM1 and CA 494 have comparatively better sensitivity and specificity compared to CA 19-9. Span-1 has comparatively better sensitivity but less or equal specificity compared to CA19-9. MIC-1 has comparatively better sensitivity but less specificity compared to CA 19-9. sTRA has comparatively better sensitivity and equal specificity, also it is secreted by pancreatic adenocarcinoma cells which are different from those which secrete CA19-9. There is a possibility that a combination of these serum markers in screening tests may yield more sensitive and specific results that can be used for screening high risk as well as the general population.

Key Words: serum tumor markers, pancreatic adenocarcinoma, pancreatic cancer screening, CA-19-9, THBS2, MIC-1, sTRA, CEACAM1, CA 494, Span-1, PAM4

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Introduction & Background

Pancreatic adenocarcinoma is not the most frequent cancer, but it is one of the deadliest cancers. The 5-year survival rate is 9% only [1]. It is mostly asymptomatic in earlier stages therefore it is usually diagnosed at a later stage when surgical resection is not possible and metastases have already spread to other parts of the body. Developing early screening tools can help in diagnosing pancreatic cancer at initial and earlier stages when surgical resection is still a possibility leading to more years of survival and a potential cure. Because of less than 100% sensitivity and specificity, screening tests are mostly not recommended for the general population, although they are recommended for high-risk populations. But there is a possibility that a combination of these tests may yield more sensitive and specific results that can be used for screening general population.

In one study, follow-up of 77 patients with pancreatic carcinoma in whom the tumor was resected showed a 100% 5-year survival rate of patients with tumor limited to the duct epithelium. The majority of these tumors were <1 cm [2]. In another study, 3 patients had 1A stage tumors <1 cm; their median survival time was 30.0 months and their 5-yr survival rate was 50.0% [3]. Based on people diagnosed with pancreatic cancer between 2009 and 2015, the combined 5-year survival rate was 9% but the 5-year relative survival rate for localized pancreatic cancer was 37% [4]. Therefore, early screening is important to detect early-stage and small-sized cancer in asymptomatic patients.

More than 90% of pancreatic cancer measuring 1 cm or less in diameter do not demonstrate any specific symptoms [5]. In one case study, a 65-year-old female patient presented to hospital without any symptoms, after a private practitioner identified a rise of her serum CA19-9 level; which was examined upon her request, without any original symptoms [5]. There was a moderate increase in tumor marker CA19-9 52 U/ml (normal range: 0-37 U/ml). No signs of pancreatitis or cholangitis were detected, at the time, the main pancreatic duct dilatation of 3.0 mm was observed by CT scan, no method of imaging was able to detect any malignant lesion After the clinical examinations and diagnostic imaging, she returned to the private practitioner for her CA19-9 levels to be monitored on a regular basis. The patient again consulted the hospital, because her serum CA19-9 rapidly increased during the last 11 months over a 29 months-period observation. Upon presentation, her CA 19-9 levels were raised to 255.3 U/ml. CT and EUS demonstrated one solitary lesion measuring 9.5 mm at the pancreatic body superior to the portal vein. Consequently, the mass was diagnosed as an adenocarcinoma of the pancreas using brush cytology from the ERCP. The tumor had not infiltrated the portal vein, the superior mesenteric artery or vein, or the coeliac plexus of nerves, and was without metastases. Therefore, a normal pancreaticoduodenectomy was performed without vesselplasty. Pathological

examination for the pancreas revealed a Stage IA invasive ductal carcinoma. The immunohistological examination also confirmed that the tumor firmly expressed CA19-9. Her clinical course was stable without complications, and she was discharged after 14 days of hospitalization. When the case was presented, the patient was systemically well, without any signs of tumor recurrence 12 months post-operatively [5]. This case shows the importance of early testing through serum Tumor markers.

In one study, a series of 177 patients with PAC treated surgically were reviewed retrospectively. A preoperative CA 19-9 level >228 U/mL, tumor size >3.1 cm, and the presence of pathological preoperative lymph nodes statistically correlated with early recurrence. Together, these three factors predicted the possibility of an early recurrence with 90.4% accuracy [6]. In another study, Tumor exceeding 4 cm, lymph node involvement, and presence of interstitial invasion are high-risk factors of recurrence after Whipple's procedure and extended radical operation. Recurrence occurred in 75% patients with tumor large than 4 cm, in 87.5% patients with lymph node involvement, and in 50% patients with the presence of interstitial invasion [7]. In yet another study, Retrospective data was gathered from 64 consecutive pancreatic adenocarcinoma patients undergoing curative Whipple procedures from 2007-2011. Patients were divided into early recurrence within 185 days, and late or no recurrence. Disease-free survival after 6 months was 64%; at 8 months, it was 50%. Factors that contributed to early recurrence included increased age at surgery, larger tumor size on pathology, and resection margins less than 0.1mm. Larger tumor size as measured by endoscopic ultrasound showed a trend for early recurrence. The study concludes that Aggressive screening and detection mechanisms for pancreatic carcinoma should be applied more frequently especially in increased age people to ensure identification of pancreatic carcinoma before large growth in tumor size [8].

Although there are many pancreatic carcinoma tumor markers for example CA19-9, CA50, CA242, CA195, CA125, CA494. PAM4, TAG-72, CEA, POA , TPA , TPS , Du-PAN 2 ,SPan-1, CAM17.1, TATI, Elastase-1, GT II , Tu M2-PK, AFP, EPM-1, OPN, MSLN, SNCG, CEACAM1, Mic-1, THBS2, sTRA but most of them do not improve upon CA19-9 which is considered a gold standard pancreatic carcinoma serum marker although CA 19-9 also does not have perfect sensitivity and specificity. Here we present a brief review of CA 19-9 and some other pancreatic adenocarcinoma serum tumor markers which show some improvement over CA19-9.

Review

CA 19-9

CA 19-9 is a predominantly carbohydrate antigen. The CA 19-9 is currently the "gold" standard marker for pancreatic cancer, against which other assays in this field are

judged [9]. According to a meta-analysis, the median sensitivity of CA 19-9 for diagnosis is 79 (70-90%) and median specificity 82 (68-91%) [10]. CA 19-9 elevation in non-malignant jaundice results in a fall in specificity. Combination with other markers improves its accuracy [10]. This tumor-associated marker is also helpful in predicting unresectability of pancreatic adenocarcinoma, as 96% of tumors that result in blood levels greater than 1000 U/ml have been found to be unresectable [9]. After potentially curative surgery, the CA 19-9 can help in giving prognosis about survival. Patients who normalize their CA 19-9 postoperatively live longer than those who do not. Furthermore, the assay, when used serially, predicts recurrence of disease prior to radiographic or clinical findings [9].

Thrombospondin 2

Thrombospondin 2 (THBS2) is a glycoprotein which belongs to Thrombospondin family. A phase 1 study on 20 subjects, a phase 2a study on 189 subjects, and a phase 2b study on 537 subjects including patients with various stages of PDAC, individuals with benign pancreatic disease, and healthy control with enzyme-linked immunosorbent assay (ELISA)using plasma samples revealed that concentrations of plasma thrombospondin-2 (THBS2) discriminated among all stages of PDAC consistently [11]. The concordance statistic was 0.76 in the phase 1 study, 0.84 in the phase 2a study, and 0.87 in the phase 2b study. The plasma concentration of THBS2 was able to discriminate resectable stage I cancer as readily as stage III/IV PDAC tumors. THBS2 plasma concentrations combined with those for CA19-9, a previously identified PDAC marker, yielded a c-statistic of 0.96 in the phase 2a study and 0.97 in the phase 2b study. THBS2 data improved the ability of CA19-9 to distinguish PDAC from pancreatitis. With a specificity of 98%, the combination of THBS2 and CA19-9 yielded a sensitivity of 87% for PDAC in the phase 2b study. A THBS2 and CA19-9 blood marker panel measured with a conventional ELISA may improve the detection of patients at high risk for pancreatic ductal adenocarcinoma [11]. With a THBS2 concentration cutoff of 42 ng/ml, THBS2 could discriminate PDAC patients from healthy primary care controls with a specificity of 99% and a sensitivity of 52%. Impressively, combining CA19-9 (>55 U/ml) with THBS2 (>42 ng/ml) showed a specificity of 98% and a sensitivity of 87% in the larger phase 2b study [11].

Macrophage Inhibitory Cytokine 1

The macrophage inhibitory cytokine-1 (MIC-1) is a divergent member of the transforming growth factor-β (TGF-β) superfamily [12]. In a study, preoperative serum from 50 patients was collected with resectable pancreatic adenocarcinoma, as well as sera from 50 patients with chronic pancreatitis and 50 age/sex-matched healthy controls [13]. Sera were analyzed for the following candidate markers of pancreatic cancer: CA19-9, macrophage inhibitory cytokine 1 (MIC-1), osteopontin, tissue inhibitor of metalloproteinase 1, and hepatocarcinoma-intestine-pancreas protein levels. In

differentiating patients with resectable pancreatic cancer from controls, serum MIC-1 was better than all other markers including CA19-9 [13]. MIC-1 was significantly better than CA19-9 in differentiating patients with pancreatic cancer from healthy controls, but not in distinguishing pancreatic cancer from chronic pancreatitis [13].

sTRA (sialylated Tumor-Related Antigen)

The sTRA glycan is a carbohydrate antigen produced and secreted by pancreatic tumors cells that are different from those that produce and secrete CA19-9. In one study, two biomarker panels improved upon CA19-9 in the training set, one optimized for specificity, which included CA19-9 and 2 versions of the sTRA assay, and another optimized for sensitivity, which included 2 sTRA assays [14]. Both panels achieved statistical improvement over CA19-9 in the validation set, and the specificity-optimized panel achieved statistical improvement in the blinded set: 95% specificity and 54% sensitivity (75% accuracy), compared with 97%/30% (65% accuracy). Unblinding produced further improvements and revealed independent, complementary contributions from each marker [14]. The sTRA and CA19-9 glycans define separate subpopulations of cancer cells and could together have value for classifying subtypes of pancreatic adenocarcinoma [15].

CEACAM1

CEACAM1 is a member of the carcinoembryonic antigen (CEA) family. In one study, CEACAM1 was expressed in the sera of 91% (74/81) of pancreatic cancer patients, 24% (15/61) of normal patients, and 66% (35/53) of patients with chronic pancreatitis, with a sensitivity and specificity superior to CA19-9. The combination of CEACAM1 and CA19-9 had significantly higher diagnostic accuracy than CA19-9 [16].

CA 494

In 59 patients with ductal pancreatic cancer the monoclonal antibody (MAb) BW 494, which detects the CA 494 glycoprotein antigen, was analyzed in comparison with the reference tumor markers CA 19-9 and CEA [17]. Eighty-one patients with non-pancreatic malignancies of the gastrointestinal (GI) tract, 95 with chronic pancreatitis, 124 with benign non-pancreatic GI diseases, 30 with diabetes mellitus (type I or type II) and 114 healthy blood donors served as controls. The sensitivity of pancreatic cancer was 90%, 44% and 90% for CA 19-9, CEA and CA 494, respectively. In chronic pancreatitis, as the most important control population for pancreatic cancer, the specificity was 85%, 72% and 94% for CA 19-9, CEA and CA 494, respectively [17].

Span-1

Span-1 is a monoclonal antibody produced by immunization to mucin-producing human pancreatic cancer cell line SW1990 [18]. In a study, Levels of serum Span-1, were tested in 64 patients with pancreatic cancer, 90 with non-pancreatic cancer, and 254 with non-malignancies, involving 55 healthy controls [19]. Frequency of elevated Span-1 levels was 81.3% in pancreatic cancer. False-positive elevations of serum Span-1 levels were rather common in liver cirrhosis (53.8%) and chronic hepatitis (26.3%). The sensitivity, specificity, and efficiency of this assay for pancreatic cancer, was 81.3%, 75.6%, and 76.5% against all subjects without pancreatic cancer, respectively. In comparison with other markers, sensitivity of Span-1 tended to be highest with similar specificity to those of CA19-9 and CEA. The Span-1 assay has a comparatively higher sensitivity than CA 19-9. However, this assay is not specific for chronic liver diseases [19]. In another study, Sensitivities of Span-1, CA 19-9, and Dupan-2 for pancreatic cancer were 94%, 85%, and 38% respectively [18]. Span-1 in patients with Stage I pancreatic cancer showed a 50% positive rating but CA 19-9 and Dupan-2 showed only 0% and 25%. Although a positive rating of these three antibodies increased in advanced cases, Span-1 showed the highest positive rating [18].

PAM4

PAM4 is a carbohydrate antigen. In a study group of 68 carcinomas, 29 chronic pancreatitides, and 19 healthy volunteers, 82% sensitivity and 95% specificity were observed with increasing sensitivities at advanced tumor stages with a sensitivity of 62% at stage I, 86% at stage II, and 91% at advanced stages 3 and 4 [20]. In another study, the presence of PAM4-reactive MUC1 is examined as a serum marker for pancreatic cancer in a total of 283 subjects, including 53 pancreatic cancer patients, 87 pancreatitis patients, 100 other cancer patients, and 43 healthy volunteers. with a sensitivity of 77% and a specificity of 95%. none of the healthy specimens and only four of 87 pancreatitis patients (5%) were positive above a cut off of 10.2 units/mL. However, of the 87 pancreatitis samples, the positive rate of CA19-9 was 37% [21]. In another study, PAM4 was tested in a study group of 298 pancreatic ductal adenocarcinomas (PDAC), 99 other cancers, 120 benign pancreases, and 79 healthy controls with 76% sensitivity and 96% specificity [22]. The specificity was significantly greater for the PAM4 assays than CA19-9 assays, particularly with regard to chronic pancreatitis (86% and 68%, resp.). The sensitivity at stage I disease was 64% and for patients with advanced disease it was 85%. Combination of PAM4 and CA19-9 I obtained an improved sensitivity (84%) for the overall detection of PDAC without a significant loss of specificity (82%) compared with either assay alone in 474 specimens [22].

Conclusions

CA 19-9 is considered the standard pancreatic adenocarcinoma serum tumor marker but there are other pancreatic adenocarcinoma serum tumor markers with

comparatively better sensitivity and specificity. THBS2 has comparatively less sensitivity but better specificity compared to CA 19-9. PAM4 has comparatively less or equal sensitivity but better specificity compared to CA 19-9. CEACAM1 and CA 494 have comparatively better sensitivity and specificity compared to CA 19-9. Span-1 has comparatively better sensitivity but less or equal specificity compared to CA19-9. MIC-1 has comparatively better sensitivity but less specificity compared to CA 19-9. sTRA has comparatively better sensitivity and equal specificity, also it is secreted by pancreatic adenocarcinoma cells which are different from those which secrete CA19-9.

Regular screening of pancreatic adenocarcinoma through tumor serum markers testing can help in earlier detection of pancreatic adenocarcinoma when its size is small and the tumor is at 1A or other earlier stage and therefore surgical resection is possible which is mostly done through Whipple procedure. But although surgical resection is termed as a potential cure for pancreatic cancer, it mostly does not cure pancreatic cancer and most of the time, pancreatic cancer recurs after resection. But the rate of recurrence for people with stage 1 A and smaller size cancer is comparatively slow and their median survival time is more especially if they are taking adjuvant chemotherapy as well. So, in the current scenario, screening cannot completely prevent pancreatic adenocarcinoma related mortality but it can lead to comparative prolongation of life for pancreatic cancer patients. With the future advancement in treatment, there is a probability that it would be comparatively easier to prevent pancreatic adenocarcinoma related mortality in earlier-stage pancreatic adenocarcinoma compared to late-stage pancreatic adenocarcinoma, so even then, tumor markers screening will remain important. Pancreatic tumor serum markers testing can be a simple, non-invasive screening test for high-risk as well as general population but because none of the serum markers including the gold standard CA19-9 is 100% sensitive and specific, so there can be a possibility of false-positive or false-negative results. The use of multiple serum markers, especially those which improve CA 19-9 sensitivity and specificity can make these screening tests more sensitive and specific and may increase the screening tests validity and reliability.

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