Tumour Markers in Pancreatic Carcinoma

**Tumour marker in pancreatic carcinoma**

**INTRODUCTION**

The incidence of pancreatic tumours has been steadily increasing almost everywhere, and has now been estimated as the fifth main cause of death in the USA and the sixth in Italy. The incidence rates, as they appear in the data of the Italian Cancer Registers, show standardized values ranging from between five and 15 per 100,000 [3] in the industrialized district of Varese in Northern Italy, the closest Regional Cancer Register to our Institution, the incidence has increased from 8.2 and 7 per 100,000 men and women, respectively, to 14.6 and 9.7 between 1977 and 1987. (Lockhart et al., 2001)

Despite the improved sensitivity of new radiological techniques to detect pancreatic cancer during the last two decades, Warshaw and colleagues [6] have underlined the fact that pre-operative imaging (CT, MRI and Angiography) has a sensitivity of only 78%. As a result, diagnosis is delayed and survival rates ranging from 1% to 5% have remained unchanged, even in resected cases, over the last three decades. There has, therefore, been a renewed interest in the role of serological markers as a diagnostic test for pancreatic tumours, because a number of mucin-associated carbohydrate antigens and enzymes are elevated in the sera of pancreatic cancer patients. (Argani et al., 2001)

This potential role of serological markers is not only limited to early detection, but may also be of assistance in the difficult clinical dilemma of differentiating pancreatic cancer from acute or chronic pancreatitis. Furthermore, serological markers of pancreatic cancer might be used in following-up patients after treatment, particularly after surgical resection, in
order to select those with minimal disease likely to respond better to medical treatment and/o radiotherapy. The optimal serological marker should be relatively cheap, easy to perform and should have high sensitivity and specificity. The literature on this topic is scanty and confusing, and mainly focused on CEA and CA19.9; only a few issues have recently been published dealing with clinical practice. (Iacobuzio-Donahue et al., 2003)

This review examines the role of common serological markers as well as new ones in the detection and follow-up of patients with adenocarcinoma of the pancreas. Markers are divided into two main categories: (i) circulating glycoproteins, such as CEA, CA19.9, CA50, CA195, CA125, CA242, SPAN-1 and others (DU-PAN-2, POA, CA494, PCAA, LAI, MSA and CASAI, and (ii) proteins with enzymatic activity including Elastase 1, phospholipase A-2 and others, such as amylase, isoamylase, alkaline phosphatase, fucosyltransferase, ribonuclease, lipase, trypsinogen and GTII. (Maitra et al., 2003)

This article assesses the role of commonly used serological markers in the detection of pancreatic cancer and follow-up. It also reviews the impact of more recent markers and compares the influence that they may have in differentiating and detecting pancreatic adenocarcinoma with a traditional policy.

CEA

The CEA antigen is an heterogeneous group of glycoproteins containing 50-80% carbohydrates and a single protein chain of about 800 amino acids. The value of CEA is well established in the diagnosis and follow-up of colorectal carcinoma, but several reports from the mid-1970s describe an increase in CEA levels in the sera of patients with carcinoma of the pancreas. It is also known that CEA levels are an accurate reflection of disease progression, as it decreases
after surgical resection in this population. Kaiser et al. reported on the relationship between circulating CEA levels and stage of pancreatic cancer: patients with localized disease had a mean level of 19±29 ng ml⁻¹ (mean ± SD); those with locally invasive/unresectable tumours a value of 26±52 ng ml⁻¹, while those with distant metastases had values of 97 ± 194 ng ml⁻¹. However a wide range of plasma levels (0-1000 ng ml⁻¹) was present in this study, as well as a high false positive rate (20%), even among subjects with advanced disease. (Swartz et al., 2003)

Despite this problem, the usefulness of plasma CEA levels in staging pancreatic carcinoma, as well as assessing its prognosis, has been well documented. A significant difference between high or low pre-operative CEA levels has been reported. One of the crucial areas in the usefulness of serological markers relates to the cut-off points used. CEA has also shown a low specificity (SP) ranging from 30 to 50% when a low cut-off point is used (2.5 ng ml⁻¹), which corresponds to 75-90% sensitivity (SS). When the cut-off is raised to 50 ng ml⁻¹, SP is increased to 80%, but SS is decreased to only 36.5-46.5%. (Hoos et al., 2001)

Reasons underlying these unsatisfactory results are twofold. The first is that CEA production in pancreatic cancer is not directly linked to tumour burden but is influenced by such factors as tumour differentiation, invasion of tissue by cancer cells, stage of the disease (local, regional, metastatic) and obstruction of the pancreatic duct. The second reason is that CEA secretion is variable depending on the degree of Kupffer cells impairment, liver functional reduction replacement by tumour or concomitant benign disease) and bile duct obstruction. (Iacobuzio-Donahue et al., 2003)
Colon specific antigen, a predominantly carbohydrate antigen, was the initial name given to CA19.9. It was defined from the culture medium of a colorectal cancer cell line SW 1116 in 1979. Koprowski and colleagues were able to distinguish patients with gastrointestinal adenocarcinomas from a normal population using a binding inhibition assay for CSA. (Maitra et al., 2003)

The CSA assay was improved by a radioimmunometric method using a monoclonal antibody named 1116 NS 19.9, hence the CSA was renamed CA19.9. In 1984, Ritts examined circulating levels in a large series of more than 1200 normal and pathologic subjects, and fixed the normal cut-off value at 37 U rnr'. Steinberg et al. increased their cut-off to 75 U rnr' in their series; this latter value raised the SP of determining pancreatic cancer by 9%, with only a 5% loss in SS. a finding in keeping with other series. It is also interesting to note that CA19.9 was not increased among smokers, and that serum levels were slightly increased in females compared to healthy male subjects. It has recently been shown that elevated serum concentrations of CA19.9 decrease after curative surgery and conversely that recurrent disease is often associated with an increase in the circulating level of this serum marker. (Argani et al., 2001)

A large number of clinical reports show a high median level of SS in pancreatic cancer patients, averaging 80% (range 68-92%). with an 84% SP; both these values are satisfactory but not sufficiently good to propose CA19.9 serum levels as the only means for detecting pancreatic carcinoma. The most useful role of this marker is in differentiating pancreatic cancer from chronic or recurring pancreatitis. It has been demonstrated that, although plasma levels are markedly increased in patients with biliary sepsis. high bilirubin levels are not usually responsible for the observed rise in serum levels. However, caution is
recommended when evaluating high serological levels in patients with jaundice of unclear origin. These same authors also compared circulating levels of CA19.9 in pancreatic cancers (100 patients) to acute and chronic pancreatitis (105 patients): SS and SP were 46 vs. 83 and 92 vs. 97 in CEA and CA19.9 respectively. (Maitra et al., 2003)

The conclusion to be drawn is that CA19.9 is a sensitive and specific marker in the differential diagnosis of pancreatic carcinoma from chronic pancreatitis. The greatest value of high CA19.9 levels (>500-1000 U ml⁻¹) is that they are almost exclusively related to malignancy and that metastatic or locally advanced disease is often associated with high levels, in keeping with the experience of Malesci et al., who noted that CA19.9 levels exceeding 120 U ml⁻¹ yield a 100% positive predictive value in non-jaundiced patients. As previously reported by Tempero et al and confirmed by Tian et al and von Rosen et al., patients with negative Lewis blood type group only express CA19.9 antigen in 40% of cases because of lack of the enzyme fucosiltransferase. For this reason, CA19.9 should not be used as a serological marker in this group of patients (which represent approximately 10% of the general population). (Lockhart et al., 2001)

CA50

The antigen CA50 was identified by Lindholm et al. in 1983 and initial studies demonstrated a number of similarities with CA19.9: (a) the antigenic determinant recognized by Mab C50 has a sialosilfucosil-lactotetraose structure which corresponds to the Lewis antigen identical to that of CA19.9; (b) similar to CA19.9, it may present as a glycolipid or high molecular weight glycoprotide; (c) it was detected in a number of epithelial tumours as well as in
normal adult pancreatic, gall bladder and gastric tissue. (Iacobuzio-Donahue et al., 2003)

It may be concluded that Mab C50 and 19.9 recognize the same antigen. Some exceptions are nevertheless evident, such as its larger distribution among different cancers, and its lower bias rate in Lewisa a-b-blood groups. This is due to the fact that it recognizes not only the sialylated Lea-antigen, but also at least one other carbohydrate structure that differs from the CA19.9 antigen by the loss of one fucose residue-this seems to be the only advantage of CA50 over CA19.9; CA50 is thus an alternative to CA19.9 among Lewisa-a-blood subjects with suspected pancreatic adenocarcinoma. Significantly increased CA50 values were observed in 41-53% of patients with benign extrahepatic cholestasis, hepatocellular jaundice or gallstone disease. (Hoos et al., 2001)

The mechanism by which cholestasis increases CA50 serum levels is still obscure, but it has been noted in subjects both with and without cholangitis. It was therefore proposed that inflammation or stasis could damage the biliary and pancreatic ducts, with the release of tumour antigens from the epithelium. (Swartz et al., 2003)

CA 195

In 1987 Bray et al. described a monoclonal antibody, named CC3C195, which identified the antigen CA195 and which does not cross-react with CEA. Serum levels of CA195 are measured by an isosandwich-type immunoradiometric assay (IRMA) and the radioiodinated antibody CC3C195 is used simply as a tracer. Despite its low reactivity with normal serum mucins, it is accepted that CA195 is a sensitive marker for patients with colon, gastric and pancreatic carcinoma. (Kallioniemi et al., 2001)
Normal control subjects show a median concentration of 2.5 U ml\(^{-1}\) and its cut-off value has been arbitrarily put at 9.0 U ml\(^{-1}\): its SS among patients with pancreatic cancer is \(~70\%\) and its SP can vary between 50 and 85\%. Cross-reactivity between CA195 and CA19.9 has been noted, and their association does not increase SS, while a dramatic increase in SS has been reported by combining CA195 and CEA. Haemolysis, hyperbilirubinaemia and lipaemia do not affect CA195 levels. \(\text{(Argani et al., 2001)}\)

**CA242**

As seen so far, most monoclonal-antibody defined serological tumour markers belong to the mucinous type of glyco-proteins. As an example, the CA19.9, CA50 and CA125 assays utilize the same antibody for detecting the antigen. Characterization of other epitopes on the carrier antigen may therefore lead to the development of assays with better clinical performance. The CA242 marker is a sialylated carbohydrate antigen co-expressed with CA50 on a mucinous type antigen called CanAg and situated on the same macromolecule. It differs from CA50 since it does not cross-react with either Lewis\(^b\) or sialylated-lactoN-tetraose\(]\). \(\text{(Iacobuzio-Donahue et al., 2003)}\)

CA242 was first isolated by Lindholm \textit{et al.} in 1985; the mean (±SD) value among normal subjects is 9.7±4.4 U ml\(^{-1}\), and a cut-off level for clinical practice is generally fixed at 20 U ml\(^{-1}\). Although some researchers noted that CA242 was increased in subjects with benign biliary obstruction, a careful prospective evaluation by pfelsson \textit{et al.} established quite clearly that jaundice might be responsible for the increase in this marker. This does not however impair its diagnostic value in the detection of pancreatic tumours, since its levels are disproportionally higher in patients with cancer. \(\text{(Hoos et al., 2001)}\)
Increased levels are related to neoplastic disease, tumour size, and stage. The sensitivity ranges between 65 and 79%, while a specificity level of 90% corresponds to a cut-off value of 39 U ml⁻¹. Pasanem et al. evaluated 193 subjects by receiver-operating characteristic-ROC curve analysis and concluded that CA242 was significantly more sensitive than CEA and CA50 at high SP levels (> 0.90). Rothlin et al. reported on a series of 300 patients and 30 healthy controls and concluded that CA242 does not improve the SS reached with CA19.9 and CASO, but that a combination of these tests does achieve both a higher SS and SP. It has recently been demonstrated that CA242 has a higher specificity over CA19.9 when a 20-30 U ml⁻¹ cut-off is used. These preliminary results must be considered with caution as they are based on a small series of patients. (Kallioniemi et al., 2001)

**SPAN-1**

SPAN-1 is a high molecular weight glycoprotein recognized by a murine monoclonal antibody produced against a human pancreatic cell line. Its epitope includes a sialic acid-like CA19.9, and a sandwich radioimmunoassay has recently been developed and clinically tested for this marker. Kiriyama et al. reported on 64 cases of pancreatic carcinoma, 90 non-pancreatic malignancies and 199 patients with benign disease where 81.3% SS and 75.6% SP rates were recorded for malignancy, and these results are in keeping with the large experience recently published by Satake & Takeuchi. (Lockhart et al., 2001)

The association of SPAN-1 with other tumour markers is reported to increase SS only to 84% when combined with CA19.9, 86% with CEA and 83% with DU-PAN-2. Chung et al have reported a sensitivity of 93% for pancreatic cancer. Mean values (± SD) in healthy controls are reported to be 6.0± 7.9 U ml⁻¹, with no significant difference between males and females. The upper cut-
off level is set at 30 U ml\(^{-1}\). Its clinical role still has to be confirmed although preliminary experiences are exciting in differentiating pancreatic cancer from chronic pancreatitis (SS = 12\%) and acute pancreatitis (SS = 26\%); the frequency of elevated serum SPAN-1 levels was high in chronic hepatitis (26\%) and liver cirrhosis (53.8\%); thus the marker must be evaluated with particular care when tested in patients with inflammatory diseases. Nevertheless the frequency of elevated serum SPAN-1 levels in pancreatic cancer was significantly higher than pancreatic or non-pancreatic inflammatory disease (81 \% vs. 15\% or 26\%). *(Skacel et al., 2002)*

**DU-PAN-2**

The monoclonal antibody DU-PAN-2-raised against a human pancreatic adenocarcinoma cell line (HPAF)-has been shown to recognize an oncofoetal and/or developmental type antigen. This antigen is present on ductal epithelial cells derived from the embryonic foregut as well as in the cells of many adenocarcinomas derived from these secretory epithelial cells. *(Swartz et al., 2003)*

It has recently been reported that the real epitope of DU-PAN-2 is LSTa (sialyllact-N-tetraose), the precursor of CA19.9, and that LSTa is accumulated in the sera of patients with the Lewis-negative phenotype; this explains why it was also found that the DU-PAN-2 assay provides complementary method for patients with Lewis-negative and CA19.9-negative subjects suspected of having pancreatic cancer. DU-PAN-2 is easily detectable in the body fluids (serum, bile, ascites) of patients with adenocarcinoma of the pancreas and might provide useful information in monitoring the course of the disease. A 55 of 68\% was initially reported by the group of researchers who developed this antigen; this figure has been confirmed by Kiriyama *et al.* while Mahvi *et al.* reported even
better values for 55 (90%) and 5P (99%). Interestingly a large recent series matching six different markers confirms the high 5P (85.3%, the highest reported), sided by the lowest 55 (47.8%). (Kallioniemi et al., 2001)

**ELASTASE-1**

This pancreatic proteolytic enzyme hydrolyses the scleroprotein elastin and plays a key role in the pathophysiology of acute pancreatitis. It is produced by the pancreatic acini and is present in the pancreatic juice as proelastase which is activated by plasminogen; for this reason it may be increased in necrotizing pancreatitis. A transgenic mouse model provides evidence that anaplastic carcinomas and islet cell tumours may arise from primitive cells that express the elastase gene, yet retain the potential to differentiate as islet cells. (Argani et al., 2001)

Firstly isolated by Banga in 1958, several technical improvements have occurred which now allow this test to be performed in 4 h thereby enhancing its clinical usefulness, particularly in emergency surgery departments. It is helpful in the diagnosis of acute pancreatitis as it is the only protease with the specific capacity of hydrolysing elastine, an important element in the structure of vascular walls. The average value in normal sera is approximately 40-fold greater than the minimal detectable amount if the cut-off value has been fixed at 400 ng dl-1. (Skacel et al., 2002)

The first important feature of Elastase-1 is its high diagnostic capacity among small pancreatic cancers. A sensitivity of 61-100% has been reported but a SP of 91-98% makes this marker a unique tool in the detection of pancreatic cancer. It has been reported that very high levels (more than 1300 ng or ') have been recorded in Stage I tumours, surprisingly exceeding the values detected in Stage II-III patients; this difference reached statistical significance in a recent
report by Scaramuzzi et al. Secondly, a pre-operative Elastase-1 test might effectively address diagnosis since chronic pancreatitis and primary liver/biliary tumours have low circulating values (approximately 300 ng dl). The reported differing serological levels according to the site of the disease (pancreatic head or tail) has not been confirmed by other authors the marker is obviously increased in the case of recurrent pancreatitis but jaundice does not seem to affect its diagnostic capacities. Finally, Elastase-1 has a short half-life, which means that a 2-week period after surgery is enough to record its basal level after tumour removal, when it is to be employed in following-up the patients post-operatively. (Lockhart et al., 2001)

OTHERS

A number of other substances have been associated with pancreatic cancer but their clinical role is not yet confirmed. These include PDA (pancreatic oncofoetal antigen) derived from the immunization of rabbits with human foetal pancreas, PCAA (pancreatic cancer associated antigen), LAI (leukocyte adherence inhibition), CAM 43, VPAN-1. P-PLA-2, CA15.3 and fucosyltransferase. (Rubin et al., 2001)

CA125 is a cancer test based on the monoclonal antibody DC125, which was originally raised against an ovarian cancer cell line. At present it is mainly utilized in the diagnosis and follow-up of ovarian carcinomas, where it has increased in 89% of the patients, but some authors have also reported an increase in pancreatic cancer. A S5 of 45-59% is reported and the SP is also quite low (76%); the association with other markers increases the S5 by only 6%. CA494 has been recently tested on a small sample of cancer patients, providing a 90% S5, but it proved more effective than CA19.9 only in detecting Stage IV-UICC patients. (Swartz et al., 2003)
The role of the above mentioned markers is currently under evaluation and their clinical role is presently not recommended as they offer no advantage over the use of CA19.9, CA242, SPAN-1 or Elastase 1. The SS of these markers never exceeds 70% and the SP never exceeds 40%. Most authors agree that the SS of pancreatic enzymes is usually lower than 60%. (Kallioniemi et al., 2001)

CONCLUSION

Four groups of tumour-related substances have been taken into account: glycoproteins (CEA), mucins (CA19.9, CA195, CA242, SPAN-1, DU-PAN2), enzymes (Elastase-1) and a mixed group of other markers, the latter of which has no proven clinical benefit. CEA has traditionally been the most commonly used marker but recent cancer associated proteins have much better SS and SP. (Skacel et al., 2002)

Among the large group of mucins, CA50 has been replaced by CA19.9 and CA195 which have a better SS and SP. The interesting results achieved by using SPAN-1 or CA242 are preliminary and need further confirmatory trials. Moreover, the use of several markers belonging to the same mucin group will possibly increase SS by only 5%, while reducing SP. Serological markers are important instruments in the follow-up of resected patients, and their association with US may increase both SS and SP. Better results are likely when CA19.9 or CA195 are matched with Elastase-1, a promising marker with a high SS and excellent SP, and which belongs to the enzyme group. Combinations of new markers hold new hope in the detection of early pancreatic cancer in high risk groups such as those with chronic pancreatitis or newly diagnosed diabetes. (Rubin et al., 2001)