Carbohydrate markers of pancreatic cancer

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Abstract
Pancreatic cancer is the fourth most common cause of death from cancer in the world and the sixth in Europe. Pancreatic cancer is more frequent in males than females. Worldwide, following diagnosis of pancreatic cancer, <2% of patients survive for 5 years, 8% survive for 2 years and <50% survive for only approx. 3 months. The biggest risk factor in pancreatic cancer is age, with a peak of morbidity at 65 years. Difficulty in the diagnosis of pancreatic cancer causes a delay in its detection. It is one of the most difficult cancers to diagnose and therefore to treat successfully. Additional detection of carbohydrate markers may offer a better diagnosis of pancreatic cancer. Carbohydrate markers of cancer may be produced by the cancer itself or by the body in response to cancer, whose presence in body fluids suggests the presence and growth of the cancer. The most widely used, and best-recognized, carbohydrate marker of pancreatic cancer is CA 19–9 [CA (carbohydrate antigen) 19–9]. However, the relatively non-specific nature of CA 19–9 limits its routine use in the early diagnosis of pancreatic cancer, but it may be useful in monitoring treatment of pancreatic cancer (e.g. the effectiveness of chemotherapy), as a complement to other diagnostic methods. Some other carbohydrate markers of pancreatic cancer may be considered, such as CEA (carcinoeblastomonic antigen), CA 50 and CA 242, and the mucins MUC1, MUC2 and MUC5AC, but enzymes involved in the processing of glycoconjugates could also be involved. Our preliminary research shows that the activity of lysosomal exoglycosidases, including HEX (N-acetyl-β-d-hexosaminidase), GAL (β-d-galactosidase), FUC (α-L-fucosidase) and MAN (α-d-mannosidase), in serum and urine may be used in the diagnosis of pancreatic cancer.

Introduction
Pancreatic cancer is very aggressive and is associated with a high mortality rate, caused by the advanced stage of the neoplastic process at the time of diagnosis. Worldwide, after diagnosis of pancreatic cancer, <2% of patients survive for 5 years, 8% survive for 2 years, and <50% survive for only approx. 3 months [1].

According to IARC (International Agency for Research on Cancer) in 2000, pancreatic cancer was newly diagnosed in 217 000 people, and 213 000 died; in Europe 60 139 cases were newly diagnosed and 64 801 died [2]. According to the Polish National Cancer Registry in 2003, pancreatic cancer caused 4000 deaths, placing it at the top of the ten most common malignancies in Poland [3]. In many countries, because of the high mortality rate and tendency to metastasize, pancreatic cancer is fourth on the list of the most common causes of cancer deaths, after lung, breast and colon cancers. Age is the biggest risk factor for pancreatic cancer, and peak morbidity appears at 65 years of age [1].

Early diagnosis of pancreatic cancer is difficult, due to negligible clinical symptoms in the early stages of the disease. Prognosis in pancreatic cancer is poor due to its rapid infiltration of surrounding tissues and therefore early formation of metastases.

In the diagnosis of pancreatic disease, imaging methods and laboratory tests both play an important role. Among imaging examinations, EUS (endoscopic ultrasonography) has the highest specificity and sensitivity, and is especially useful when changes are small (less than 15 mm) [4]. Unfortunately, EUS is too expensive, requires specialized equipment and is not generally available. Therefore progress in the diagnosis of pancreatic cancer could well rely on methods based on substances (markers) produced by the cancer or by the body in response to cancer, whose presence in body fluids could indicate the presence and growth of pancreatic cancer [5]. The ideal marker for cancer would be a ‘blood test’ in which a positive result would occur only in patients with malignancy, which would correlate with the stage and response to treatment, be easily measured, and have high reproducibility [5]. Unfortunately, no currently available tumour marker has met these criteria.

Cancer cells often secrete specific glycans, which may be applied in its diagnosis. These glycans, appearing in either a free form or covalent complexes with proteins and lipids, are either membrane-associated or secreted [6]. The most
widely used and best-recognized carbohydrate marker of pancreatic cancer is CA 19–9 [CA (carbohydrate antigen) 19–9].

**CA 19–9**

CA 19–9 (sialylated Lewis blood group antigen), determined in serum, is commonly used in monitoring a response to therapy in patients with pancreatic cancer [1,7,8]. CA 19–9 is regarded as the best marker for pancreatic cancer with an 80% sensitivity and 90% specificity [9]. However, the usefulness of CA 19–9 for diagnosis and monitoring of pancreatic cancer is reduced by false-positive results in patients with biliary, hepatocellular, gastric, colonic and non-gastrointestinal malignancies [8]. In addition, some people (from 5 to 10% of the population) have no sialylated Lewis blood group antigen and are unable to synthesize CA 19–9 [10]. According to the guidelines of EGTM (European Group on Tumor Markers) [11], CA 19–9 is of limited value in the diagnosis of pancreatic cancer, especially in the early stages of its development. Experts believe that CA 19–9 may complement radiological examination, especially in non-jaundiced patients [11]. The NACB (National Academy of Clinical Biochemistry) does not recommend CA 19–9 measurement for the diagnosis of pancreatic cancer. According to NACB, CA 19–9 used alone to diagnose pancreatic cancer is insufficient and needs to be applied in combination with other examinations such as CT (computed tomography) or EUS [11]. According to the NCCN (National Cancer Comprehensive Network), “the degree of increase in CA 19–9 levels may be useful in differentiating pancreatic adenocarcinomas from inflammatory conditions of the pancreas”. The NCCN recommends caution in the use of CA 19–9 in the diagnosis of pancreatic cancer, because of false-positive findings that occur in patients with benign biliary obstruction, and false-negative findings that occur in subjects with a Lewis (a)-negative genotype [11].

The available literature suggests that determining CA 19–9 is useful in detecting recurrent/metastatic disease a few months before the appearance of clinical and radiographic symptoms of pancreatic cancer [12]. According to the NACB, when used alone, CA 19–9 does not permit a diagnosis of the recurrence of pancreatic cancer without confirmation by clinical symptoms and/or biopsy [13]. Thus increased blood levels of CA 19–9 do not necessarily indicate the presence of pancreatic cancer. Although CA 19–9 cannot be used in the diagnosis of the early stages of cancer development, it may be helpful, along with other tests, in the detection of recurrent/metastatic pancreatic cancer.

**Potential carbohydrate markers of pancreatic cancer**

**CA 50**

CA 50, a novel cancer-associated carbohydrate marker, is detected using an anti-CA 50 antibody. The antibody has been obtained by immunization of mice with a human colorectal adenocarcinoma cell line. The CA 50 antibody reacts with the afucosyl forms of sialylated Lewis (a) carbohydrate moiety and sialylated Lewis (a) moiety (which is also the antigenic epitope in the CA 19–9 assay). The CA 50 marker is not organ-specific, and its elevated levels in serum can be observed in a variety of malignancies, especially in gastrointestinal cancers. The level of the CA 50 is elevated in the serum of patients suffering from benign liver and biliary tract diseases, especially in cases of jaundice [14]. CA 50 gives fairly similar results to CA 19–9 and has little diagnostic value, but is very useful for monitoring patients with pancreatic cancer. Determination of CA 50 is characterized by a high sensitivity of 96%, but has a low specificity of 48% [15].

**CA 242**

CA 242 is a tumour marker whose structure is still unresolved; however, there is evidence that CA 242 has a sialylated carbohydrate type I chain, but which is different from that of CA 19–9 and CA 50. In serum, the CA 242 epitope seems to be located in the same macromolecular complex as CA 19–9 and CA 50 [16].

Ozkan et al. [17] compared the diagnostic value of CA 242 and CA 19–9 with CEA (carcinoembryonic antigen) in patients with pancreatic cancer, and found a positive correlation between levels of CA 242 and CA 19–9. The determination of CA 242, CA 19–9 and CEA in patients with pancreatic cancer had 75, 80 and 40% sensitivity, as well as 85.5, 67.5 and 73% specificity respectively. According to Haglund et al. [18], the determination of CA 242 is an alternative to CA 19–9 in the diagnosis of pancreatic cancer. The advantage of CA 242 over CA 19–9 is its higher specificity when using the recommended cut-off levels of the assays. The sensitivity of the CA 242 assay in the diagnosis of pancreatic cancer was 55% in stage I, 83% in stages II–III, 78% in stage IV and 74% for the overall sensitivity. In conclusion, it seems that CA 242 is a marker with greater diagnostic value than CA 19–9.

Liao et al. [19] compared CA 19–9, CA 50, CA 242 and CEA, and have shown that CA 19–9 is a highly sensitive marker (75.36%) and CEA is a highly specific marker (93.90%) for pancreatic carcinoma. They show that simultaneous determination of CA19–9, CA 242, CA 50 and CEA produced 97.80% specificity for the detection of pancreatic cancer, suggesting that levels of serum CA 19–9, CEA and CA 242 are all of clinical significance, and this combination is useful as a 'check-up' marker for differential diagnosis of solid lesions located at the pancreatic head.

**CEA**

CEA is a highly N-glycosylated (28 potential site of N-glycosylation) cell-adhesion glycoprotein of the immunglobulin superfamily linked to the apical colon endothelial cell membrane by a GPI (glycosylphosphatidylinositol) linkage. It was suggested that a decrease in the molecular mass of human colonic CEA could be related to the glycan type and
degree of glycosylation of the glycoprotein. CEA is not suitable for the screening of early colorectal cancer because of its low sensitivity in the early stages and elevation without malignancy. CEA measurement is recommended every 2–3 months for the prognosis and surveillance following curative resection, and for monitoring therapy in advanced disease. The serum concentration of CEA increases in colorectal adenocarcinoma and adenocarcinomas of the pancreas, stomach, breast, lung, genital tract and urinary bladder. Elevated serum levels of CEA are present in non-epithelial cancers (neuroblastoma, sarcoma and lymphoma) and non-malignant diseases, such as liver inflammation and cirrhosis, chronic pancreatitis, stomach ulcers and duodenal ulcers, ulcerative colitis and even in physiological states such as pregnancy [16]. Liao et al. [19], studying CA 19–9, CA 50, CA 242 and CEA, have shown that CEA is the most specific marker (93.90%) for pancreatic carcinoma. Simultaneous determination of CA 19–9, CA 242, CA 50 and CEA has a high specificity (97.80%) in the diagnosis of pancreatic cancer. A valid conclusion is that the determination of serum CEA levels, in addition to other markers, is helpful in the diagnosis of pancreatic cancer.

### Mucins as markers of pancreatic cancer

Mucins produced by various epithelial cells are high-molecular-mass glycoproteins with oligosaccharides attached to serine or threonine residues of a core protein by O-glycosidic linkages. They are categorized into membrane-associated mucins (MUC1, MUC3, MUC4, MUC12, MUC16 and MUC17), gel-forming secreted mucins (MUC2, MUC5AC, MUC5B and MUC6), and soluble and secreted mucins (MUC7) [20]. Mucins play an important role in the formation and progression of carcinogenesis and in tumour growth [11,20].

MUC1, MUC2, MUC4 and MUC5AC are possible markers of pancreatic cancer [11]. Simultaneous measurement of MUC1 and MUC5AC plus cytology has a significantly higher sensitivity and accuracy for pancreatic cancer than cytology alone. For screening, the combination of MUC2 and MUC5AC plus cytology yielded higher sensitivity and specificity for mucinous tumours than cytology alone [11].

The PAM4 antigen (pancreatic cancer MUC1) is characterized by relatively high specificity in comparison with antigens from other cancers (e.g. breast, ovarian etc.). An immunohistochemical study of normal adult tissues showed that PAM4 antigen is absent in the healthy pancreas. In neoplastic tissue, PAM4 antigen was present in 85% of the pancreatic carcinomas, approximately half of the colon cancers, and none of the breast, ovarian, prostate, renal and liver cancers. The PAM4 epitope is a conformationally dependent peptide epitope, and carbohydrate chains of certain structures are necessary to maintain the correct peptide conformation [21–24]. It seems that PAM4 antigen may be a marker in the early diagnosis of pancreatic cancer [25,26].

In summary it may be stated that some of the best-known pancreatic cancer tumour markers are mucins: CA 125 is directed to MUC16, CA 15–3 to MUC1 and the CA 19–9 assay detects a sialylated lacto-N-fucopentose II of mucin-like molecules [11].

### Lysosomal exoglycosidas in pancreatic cancer

Lysosomal exoglycosidas release single sugars from the non-reducing ends of the oligosaccharide chains of glycoconjugates [27]. The source of exoglycosidas may be white blood cells, such as macrophages, neutrophils and lymphocytes, accumulated around the tumour as a result of inflammation and damaged pancreatic tissue itself. Macrophages and neutrophils secrete lysosomal exoglycosidas into the extracellular space [28,29]. The disease changes the activity of one or more exoglycosidas. Cancer cells are capable of producing a variety of hydrolytic enzymes, including lysosomal exoglycosidas, that have the capacity to destroy the connective barrier [30,31].

Our preliminary findings have shown that the activity of the lysosomal exoglycosidas HEX (N-acetyl-β-D-hexosaminidase), GAL (β-D-galactosidase), FUC (α-L-fucosidase) and MAN (α-D-mannosidase) in serum and urine can be used in the diagnosis of pancreatic cancer [32]. In this regard, the most useful of these enzymes is the activity of the most active exoglycosidase HEX and its isoenzymes HEX A and HEX B [32,33].

We found that HEX, HEX A and HEX B in serum can be used in the differential diagnosis of thyroid, kidney and pancreatic adenocarcinomas, as in the serum of patients with thyroid cancer only HEX A increased significantly [34] and HEX, HEX A and HEX B were significantly increased in renal cancer [35,36], whereas in pancreatic cancer only HEX A and HEX [33] were significantly increased. Our results indicate the high diagnostic value of HEX, HEX A and HEX B activity in serum and urine as pancreatic cancer markers [37].

As mentioned above, the ability to detect pancreatic cancer only at an advanced stage of the disease is a major cause of death. A comprehensive study in serum and urine of the lysosomal exoglycosidas HEX, GAL, MAN and FUC, along with the markers used routinely, could be a significant step forward in the diagnosis of pancreatic cancer. Our results indicate differences in the serum and urinary activity of lysosomal exoglycosidas in patients with adenocarcinoma of the pancreas in comparison with colon adenocarcinoma [32,38], and therefore our results suggest that lysosomal exoglycosidas could be potential markers in differentiating pancreatic adenocarcinoma from colorectal cancer [32,38]. In patients with pancreatic adenocarcinoma, the serum activity of GAL decreased significantly in the absence of significant differences in the urine, whereas, in colon adenocarcinoma, serum and urinary GAL activity increased [32,38] in comparison with healthy subjects. In the differential diagnosis of pancreatic and colon cancers,
determination of urinary MAN and FUC, which have reduced activity in the urine of patients with adenocarcinoma of the pancreas, may be applied. No significant differences in the urine of patients with adenocarcinoma of the colon were found in comparison with healthy subjects [32,38]. Our published findings show that the exoglycosidase with the highest sensitivity and specificity as a marker of pancreatic cancer is HEX, both in serum and in urine. Our results suggest the possibility of using the profiles of lysosomal exoglycosidases in the differential diagnosis of pancreatic cancer [32,33].

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References


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