CA 19-9: Biochemical and Clinical Aspects

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Abstract

CA19-9 (carbohydrate antigen 19-9, also called cancer antigen 19-9 or sialylated Lewis a antigen) is the most commonly used and best validated serum tumor marker for pancreatic cancer diagnosis in symptomatic patients and for monitoring therapy in patients with pancreatic adenocarcinoma. Normally synthesized by normal human pancreatic and biliary ductal cells and by gastric, colon, endometrial and salivary epithelia, CA 19-9 is present in small amounts in serum, and can be over expressed in several benign gastrointestinal disorders. Importantly, it exhibits a dramatic increase in its plasmatic levels during neoplastic disease. However, several critical aspects for its clinical use, such as false negative results in subjects with Lewis $^{a-b-}$ genotype and false positive elevation, occasional and transient, in patients with benign diseases, together with its poor positive predictive value (72.3 %), do not make it a good cancer-specific marker and renders it impotent as a screening tool. In the last years a large number of putative biomarkers for pancreatic cancer have been proposed, most of which is lacking of large scale validation. In addition, none of these has showed to possess the requisite sensitivity/specificity to be introduced in clinical use. Therefore, although with important limitations we well-know, CA 19-9 continues being the only pancreatic cancer marker actually in clinical use.

Keywords

Biomarker • CA 19-9 • CA 19-9 biochemical structure • CA 19-9 measurement • Clinical interferences • DUPAN-2 • Follow-up pancreatic cancer • K-ras • Methodological interferences • MIC-1 • MicroRNA • Pancreatic cancer • REG-4 • Serum tumor markers • Sialylated Lewis a antigen

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15.1 Introduction

CA 19-9 (carbohydrate antigen 19-9, also called cancer antigen 19-9) is the most widely used and best validated marker for pancreatic cancer [1]. First described in 1979 by Koprowski et al. [2] in colorectal carcinoma cell line SW1116 using the mouse monoclonal antibody 1116-NS-19-9, this molecule was then discovered in the serum of patients with colon and pancreatic cancer in 1981 [3] and was later found also to be a component of glycoproteins and mucins [4–6]. It belongs to the large family of mucinous markers: glycoproteins with a transmembrane protein skeleton and the extracellular side consisting of oligosaccharides chains extensively glycosylated, which are a normal component of the glandular secretions of mucous type. In particular, CA 19-9 is synthesized by normal human pancreatic and biliary ductal cells and by gastric, colon, endometrial and salivary epithelia. Normally present in small amounts in serum, in which it exists as mucin, a high molecular mass (200–1000 kDa) glycoprotein complex, CA 19-9 is over expressed in certain inflammatory conditions as pancreatitis and other benign gastrointestinal diseases. Moreover, it exhibits an increase in its plasmatic levels in course of neoplastic disease, during which several processes regulating both the passage of these molecules in the bloodstream and their metabolization appear altered [7]. Sialyl Lewis a is not found at high levels in normal tissues, whereas it is found at elevated levels in patients with pancreatic, hepatobiliary, gastric, hepatocellular, colorectal and breast cancer.

15.2 Biochemical Structure

CA 19-9 antigen is a tetrasaccharide carbohydrate termed sialyl Lewis a (part of the Lewis family of blood group antigens) with the sequence Neu5Aca2,3Galb1,3 (Fuca1,4) GlcNAc. Sialyl Lewis a is synthesized by glycosyltransferases which sequentially bind the monosaccharide precursors onto both N-linked and O-linked glycans. The expression of the antigen requires the Lewis gene product, 1,4-fucosyltransferase, and subjects who are genotypically Le^{a-b-}, approximately 6 % of Caucasian and about 22 % of non-Caucasican population, do not synthesize the molecule. The Lewis blood group system comprises a set of fucosylated glycosphingolipids that are synthesized by exocrine epithelial cells and subsequently adsorbed onto the surface of the erythrocyte, giving rise to their Lewis phenotype and thus circulating in body fluid as red cell antigens. The Lewis antigen system is based on expression of genes members of the fucosyltransferase family, which catalyzes the addition of α-fucose residue to precursor polysaccharides in the last step of Lewis antigen biosynthesis. In particular, enzymes with α 1 → 3 fucosyltransferase and α 1 → 4 fucosyltransferase activities, encoded by Le or FUT3 gene, add an α-fucose residue to the precursor oligosaccharide substrate in subterminal position, converting it to the Le^a antigen. The α-fucose residue linked to terminal β-galactose through 1 → 2 linkage is synthesized by the α1 → 2 fucosyltransferase, encoded by FUT2 (Se) gene, and can be added only if an α-fucose has already been added by the Le gene product. Therefore, the addition of a second fucose to the Le^a antigen produces the Le^b antigen.

Besides Le^a and Le^b, also two minor antigens exist, Le^c and Le^d, and several sialylated or sulfated forms of antigens whose identification has been facilitated by the use of monoclonal antibodies, started on a large scale about 30 years ago. Le^{a-b+} phenotypes are present with a frequency of 72 % among Europeans and white American populations, followed by Le^{a+b-} (22 %), and Le^{a-b-} (6 %), while the percentage of Le^{a-b-} is as high as 22 % in Afro-Americans [8]. The Le^{a+b+} phenotype is more frequent among people of East Asia and the Pacific rim region, due to the presence of *Se* genes encoding less efficient α1 → 2 fucosyltransferase [9].

Le^a and Le^b antigens start to appear after birth, the first develops soon, the second much later, till it reach the adult level at 6 years of age. It has long been known that Le^a and Le^b glycolipid antigens are mainly synthesized by intestinal epithelial cells, secreted into the blood stream, and adsorbed at the surface of RBCs. This process can be

affected by abnormalities in serum lipoprotein composition during pregnancy or malignant disorders, thus resulting in a considerable decrease of Le^a and Le^b antigen expression on RBCs.

15.3 Physiology and Pathophysiology

It is well known that immune cells express specific recognition molecules for cell surface glycans, such as galectins, sialic acid binding Ig-like lectins (siglecs), and selectins [9–11]. Such recognition molecules seem to be essential in cell-cell interaction processes, but the exact mechanism that involve glycan-mediated cell-cell interactions in mucosal immunity are still to be clarified.

It has also long be known that cell surface glycans undergo remarkable changes during malignant transformation, an altered expression ascribable to a process already defined 'incomplete synthesis' of complex carbohydrate determinants, with the resulting expression of structurally less complicated carbohydrate molecules [12–15].

The sialyl Lewis a antigen is just one of these carbohydrate determinants. It has recently been shown that, besides this determinant, linked to a single molecule of sialic acid, there is another form, tied to two molecules of sialic acid (the second sialic acid residue attached at the C6-position of penultimate GlcNAc in sialyl Lewis a), which is prevalently expressed in non-malignant epithelial cells (disialyl Lewis a). This 'normal' molecule, whose expression decreases significantly during malignant transformation, functions as a ligand for immunosuppressive receptors and contributes to maintaining immunological homeostasis of the gastrointestinal mucous membranes. In particular, studies conducted by Miyazaki et al. [16] indicate that the glycans expressed in normal epithelial cells serves as ligands for sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) and sialic acid-binding immunoglobulin-like lectin-9 (Singlec-9), the immunosuppressive carbohydrate-recognition receptors expressed mainly on leukocytes, whereas the cancer-associated glycans do not. The downregulated transcription of a gene encoding the $\alpha 2 \rightarrow 6$ sialyltransferase in cancer cells produces initially a partial synthesis of incomplete bond of the second sialic acid residue then a gradual transition of carbohydrate determinants from disialyl Lewis a-dominant status to sialyl Lewis a-dominant status, with a resulting accumulation of this last. Important functional consequences are evident, such as the loss of right cell-cell recognition between mucosal epithelial cells and lymphoid cells and the gain of E-selectin binding activity. Similarly, impairment of 6-sulfation seems to occur on malignant transformation of colonic epithelial cells, leading to the loss of sialyl 6-sulfo Lewis x determinant and gain of sialyl Lewis x in cancer cells, another ligand for E-selectin [17]. Therefore, the expression of these siglec-7/-9 ligands that was impaired upon carcinogenesis were replaced by cancer-associated glycans sialyl Lewis a and sialyl Lewis x, which have no siglec ligand activity. If normal glycans of epithelial cells exert a suppressive effect on cyclooxygenase-2 expression by resident macrophages, thus maintaining immunological homeostasis in colonic mucosal membranes, their loss caused by impaired glycosylation can enhance inflammatory mediator production [18]. Subsequently, hypoxic conditions that arise in the course of neoplastic disease, in inducing the transcription of several genes responsible for glycosylation involved in the synthesis of sialyl Lewis a, further accelerate the expression of this determinant in hypoxia-resistant cells with a high degree of malignancy, which become the predominant clones in advanced tumors with high frequency of hematogenous metastases [19].

15.4 Measurement of CA 19-9

15.4.1 Clinical Interferences with the Assay

Initial enthusiasm for applying sialyl Lewis a for serum diagnosis of cancers has waned in part when the presence of false-positives in patients

suffering from intra- and extra-cholestatic diseases as well as liver dysfunction have been reported [20–22]. Since then, it appeared to be clear that clinical interpretation of CA 19.9 measurement requires a careful evaluation of important interfering situations which render difficult the use of this tumor marker in clinical practice. Now, in the light of more recent data analyzing the diagnostic accuracy in patients with pancreatic cancer, appear evident that the diagnostic utility of CA 19.9 presents important limitations above all related to a low sensitivity in sympthomatic patients and a low PPV. In particular for the following:

- Impossibility to detect CA 19-9 in subjects that have a fucosyltransferase deficiency, approximately of 5–10 % of the Caucasian population, who cannot synthesize the Ca-19-9 epitope. Therefore, in these genotypically Lewis $^{a-b-}$ patients, false negative results for CA 19-9 serum levels can be obtained even in the presence of advanced pancreatic cancer. It follows that the maximum achievable sensitivity of CA 19-9 for pancreatic cancer in Caucasian populations is 90–95 % [23];
- Appearance of sialyl Lewis a in the serum is not specific to malignant disorders, and patients with benign disorders sometimes show elevated serum levels of sialyl Lewis a.
- The occasional and transient elevation of CA 19.9 serum levels in a wide variety of benign conditions limits its diagnostic utility, showing as sialyl Lewis a is not a cancer-specific marker in a strict sense. The determinant is expressed by a small number of ductal epithelial cells in the normal pancreas, and its serum levels exhibit an increase, sometimes dramatic [24], in several non-malignant disorders such as inflammatory diseases, including chronic and acute pancreatitis, liver cirrhosis, cholangitis and obstructive jaundice [25]. Other benign conditions, including ovarian cyst, heart failure, hashimoto's thyroiditis, rheumatoid arthritis and diverticulitis have been reported to cause an increase of CA 19-9 serum levels [26–31];
- Possibility to detect elevated CA 19-9 levels in multiple types of adenocarcinoma, especially in advanced gastrointestinal cancers [1,7,26]. In an overview study, Steinberg [26] reported an elevation percentage, which sometimes may be significant, of CA 19-9 in patients with bile duct cancer, gastric and colorectal cancer, and with hepatocellular carcinoma;
- Lacking in CA 19-9 sensitivity for early or small-diameter pancreatic cancers. Because of serum CA 19-9 concentration is highly correlated to the tumor size in most, if not in all, patients with pancreatic cancer [32], just 50 % of patients with pancreatic cancers less that 3 cm in diameter presents elevated levels of CA 19-9 [26], thus it is difficult to use CA 19-9 as a marker for early diagnosis of pancreatic cancer [33,34];
- Poor correlation between the degree of cell differentiation of the tumor and the serum level of CA 19-9 (National health Insurance Corporation [35]). Poorly differentiated pancreatic cancers appear to express less CA 19-9 than either moderately or well differentiated cancers [26].

Given all these limitations, it is evident the CA 19-9 is a marker that should be used carefully, particularly in the initial diagnostic approach, during which its use may at worst aid diagnosis, but of course cannot replace histological proof of pancreatic cancer, even when imaging is indicative [1]. Moreover, if false-positive results in a given population of patients with benign disorders are inevitable, however the possibility to simultaneously determinate serum levels of sialyl- and disialyl Lewis a and to calculate the monosialyl/disialyl Lewis a ratio is very important to limit these false positives. In particular, during the course of cancer progression, the expression of sialyl Lewis a determinant is accelerated, with consequent increase of sialyl Lewis a/disialyl Lewis a ratio, which tends to be higher in serum of cancer patients while maintaining low in patients with benign disorders. In this way it is possible to distinguish pathological forms more severe than the benign, thus reducing the number of patients who are sometimes subjected to long hospitalization periods, and undergo unnecessary further clinical examinations,

including diagnostic imaging techniques, for have differential diagnosis.

15.4.2 Methodological Interferences with the Assay

Almost all assays for CA 19-9 detection depend on the use of the monoclonal antibody 1116-NS-19-9, which recognizes a carbohydrate epitope expressed on circulating antigen. An important aspect must be considered about CA 19.9 assays. In fact, although assays for the quantitative detection of CA 19-9 have been available for almost 30 years, its measurement is still somewhat problematic, reflecting primarily the lack of an international standard for CA 19-9 and differences in assay design. A comparative analysis of different assays for CA19-9 carried out extensively over the last few years has clearly demonstrated that different assays may give different results [36–38]. Also a recent study, undertaken to compare the results obtained by two widespread commercial methods, showed that the two assays were comparable in diagnostic accuracy and had a good correlation, but are not interchangeable [39]. The poor comparability of CA 19-9 results obtained using different methods complicates their clinical interpretation. It is therefore fundamental that patients who undergo serial determination of CA 19-9 levels are monitored for this marker using a single method and that each report states the method used for analysis [40].

Another problem in measuring of CA 19.9 is represented by the possibility of obtaining false results caused by the presence of interference methodology. Although interferences in the CA 19-9 assay are not frequent, this phenomenon, common to all immunoassays, must therefore always be considered. It has been reported that the presence of rheumatoid factor (RF) and of heterophilic antibodies are the most important causes of interference in the determination of CA 19-9. First described by Biguet et al. in [41], the possible interference of RF in the determination of CA 19-9 has been afterwards evaluated by Berth and co-workers in an RF-positive population, with RF concentrations exceeding 100 kIU/L, using four different immunoassay platforms [42]. The Authors reported that, among the eight discrepant results probably related to method dependent differences, only one, obtained with an assay for CA 19-9 (Centaur, Siemens Healthcare) but not with three others (ARCHITECT and AxSYM, Abbott, and Vidas, Biomerieux), is clearly referable to a interference problem of RF, with high level positivity for high RF (900 kU/L) associated with a very high positivity of CA 19-9 (80,000 U/L).

Contrarily, in a case report of a patient with a history of biliary polyp, Liang et al. [43] exclude that RF is responsible for the falsely elevated carbohydrate antigen 19-9 level, attributing instead this false-positivity to the presence of heterophilic antimouse antibody interference. Regarding the possible interference by heterophilic antibodies in serum CA 19-9 determination, Passerini et al. [39] demonstrated that both immunoassays considered in their study appeared to be affected by such interference, because a reduction of values below the proposed diagnostic cut-off was seen in 40–46 % of discrepant specimens after these antibodies were removed.

15.4.3 Sensitivity, Specificity

Some scientific publications have been carried out on the diagnostic accuracy of CA 19-9 in patients with pancreatic cancer and have been recently revised by Duffy et al. [1] in their exhaustive and comprehensive review. In all these works, in which serum CA 19-9 levels in pancreatic cancer patients have been compared with different control groups, has been used 37 kU/l as cut-off point for CA 19-9 and, with this cut off, CA 19-9 has been shown to have an overall mean sensitivity of 81 % and a mean specificity of 90 % for pancreatic cancer. Increasing the cut-off point improved considerably the specificity, but reduced gradually the sensitivity [26]. Data from 1990 to 2005, analyzed by Goonnetilleke and Siriwardena in a recent review, showed a median sensitivity of CA 19-9 for pancreatic cancer of the 79 % and a median specificity of 82 % [7]. Moreover, CA 19-9 sensitivity varies with the stage of pancreatic cancer, and only 50 % of patients with pan-

creatic cancers of <3 cm diameter will have an elevated CA 19-9 level. As reported in a document of the Association for Clinical Biochemistry [44], sensitivity for other malignancies is the following: 70 % for hepatobiliary, 40–50 % for gastric cancer, 30–50 % for hepatocellular carcinoma, 30 % for colorectal cancer and 15 % for breast cancer.

15.5 Clinical Indications

15.5.1 Pancreatic Cancer

According to American Cancer Society, in 2014 there will be 46,420 new cases of pancreatic cancer and an estimated 39,590 people will die of this disease. Rates of pancreatic cancer have been increasing slightly over the past decade, accounting for about 3 % of all cancers in the United States, and for about 7 % of cancer deaths. Compared to other cancers, pancreatic cancer is relatively rare, with an average lifetime risk of developing it of about 1.5 %. Although only the 12th most frequent malignancy, cancer of the pancreas was the fifth most frequent cause of cancer-related mortality in the Europe [1] and the fourth leading cause of cancer death in the US. With increasing age, this cancer type becomes more common and slightly more common in men than women.

Cancer stage at diagnosis addresses to chemotherapy or chemoradiotherapy treatment options and early detection has a strong influence on the patient survival. In general, the earlier pancreas cancer is caught, the better chance a person has of surviving 5 years after being diagnosed. The 5-year survival for localized pancreas cancer (approximately 9 % of the total) is of 25.8 %. Moreover, only 20 % of patients who have diagnosis of pancreas cancer are considered eligible for surgery and, of these, about a half undergoes successful resection. For the remaining 80 % of patients, suffering from locally advanced or metastatic disease, no curative therapy currently exists, and the median survival times estimated for them are of the order of 8–12 months and 5–8 months, respectively [1]. This poor prognosis is attributable to late pancreas cancer detection, that renders often ineffective the therapeutic treatments, to its early recurrence and, above all, to the absence of clinically useful biomarker(s) which can detect pancreatic cancer in its precursor form(s) or earliest stages [45]. Therefore, the prognosis continues to be poor, despite some improvements, mainly due to a more specialized surgery treatment and to the application of specific chemotherapy protocol, have been made in recent years. Yet, the large number of new putative pancreatic biomarkers that have been recently proposed needs to a large scale clinical validation, which at present still lacks.

15.5.2 CA 19-9 as a Screening and Diagnostic Biomarker for Pancreatic Cancer

The role of CA 19-9 as a screening tool for pancreatic cancer in asymptomatic individuals has been extensively evaluated, demonstrating that it has no utility as a screening marker given its very low positive predictive value [27,28]. In particular, Kim et al. [27] have drawn this conclusion, analyzing data from our study in which 70,940 asymptomatic subjects were screened using CA19-9. Only four cases of pancreatic cancer were detected along with 1059 false-positives, yielding a positive predictive value of only 0.9 %, although the sensitivity and specificity were 100 % and 98.5 % respectively. Similarly, Chang et al. [28], in illustrating results of our screening study on a group of 5343 subjects, reported that only two, among the 385 patients with CA 19-9 serum level >37 U/ml, were suffering from pancreatic cancer. The PPV of an elevated serum CA 19-9 level in the asymptomatic population in this study was only 0.5 %. False positive elevation of the CA 19-9 serum levels was noted in 325 patients (6.1 %) and a total of 58 other cancers were identified. Moreover, in screening high-risk populations, serum CA19-9 level is often normal also when many preinvasive pancreatic lesions are detected by imaging [46–48]. Based on these evidences, according to American Society of Clinical Oncology (ASCO) guidelines, CA 19-9

should not be used as screening in asymptomatic subjects. Currently, a multimodality screening combining various evaluative imaging techniques appears to be the most effective way to detect precancerous pancreatic lesions, even though it is an issue still controversial in some its aspects (the age to initiate screening, the optimal screening modalities as well as the intervals for follow-up imaging). In 2013, International Cancer of the Pancreas Screening (CAPS) consortium state that "initial screening should include endoscopic ultrasonography (EUS) and/or magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP), not computed tomography (CT) or endoscopic retrograde cholangiopancreatography (ERCP)" [49,50].

In addition to screening, early detection of pancreatic cancer is important for a differential diagnosis and a timely management of this malignancy. The utility of serum CA19-9 in the diagnosis of pancreatic cancer has been extensively evaluated, as well as the diagnostic cutoff value of CA19-9. Results from a study performed in 1999, enrolling 20,035 asymptomatic subjects, 160 patients with pancreatic diseases and 322 with biliary tract diseases, showed a mean serum concentration of CA19-9 in asymptomatic individuals of 9.42 ± 9.95 U/ml. Levels above 37 U/mL were determined to be most accurate for discriminating pancreatic cancer from benign pancreatic diseases (sensitivity and specificity of 77 % and 87 %, respectively) [29]. The diagnostic utility of CA 19-9 has been investigated also in the already mentioned review by Goonnetilleke and Siriwardena [7], who analyzed pooled data from 2283 symptomatic subjects. The Authors reported a median sensitivity of serum CA 19-9 level for pancreatic cancer of 79 % and a median specificity of 82 % with a PPV and NPV of 72 % and 81 % respectively. Among patients with symptoms suspicious for pancreatic cancer, elevated CA 19-9 is a poor predictor of pancreatic cancer with a predictive value of 0.5–0.9 %. Based on this evidence of poor sensitivity for early lesions, the European Group on Tumor Marker (EGTM) guidelines affirms that CA 19-9 has limited value in the diagnosis of pancreatic cancer, especially for early forms of the disease. Similarly, the National Academy of Clinical Biochemistry (NACB; USA) does not recommend measurement of CA 19-9 in the diagnosis of pancreatic cancer, but states that the marker could be used in aiding diagnosis, in conjunction with results from accurate radiological procedures, such as computed tomography (CT) or endoscopic ultrasound (EUS) and can guide further invasive testing such as endoscopic retrograde cholangiopancreatography, laparoscopy or EUS fine-needle aspiration [1].

15.5.3 CA 19-9 Serum Levels as a Biomarker of Prognosis in Patients with Pancreatic Cancer

Measuring serum CA 19-9 levels provides significant prognostic information and allows patient stratification (survival groups) and determination of resectability of pancreatic cancer. For example, based on pre-operative CA 19-9 levels, Berger et al. stratified 129 surgically resected pancreatic cancer patients into four groups [(undetectable, normal (<37 U/ml), 38–200 U/ml, and >200 U/mL)], demonstrating an inverse correlation between CA 19-9 levels and median survival of patients [51]. Preoperative CA19-9 levels ($p = 0.030$) and lymph node ratio ($p = 0.042$) emerged as independent predictors of survival on multivariate analysis conducted by Smith et al. [52] in patients with resected pancreatic ductal adenocarcinoma. Data from study of Zhang et al. [53] showed that preoperative serum CA19-9 level is a useful marker for evaluating the resectability of pancreatic cancer, while the multivariate analysis of factors predicting survival, conducted by Waraya et al. [54] in 117 pancreatic cancer individuals undergoing surgical resection, demonstrated the prognostic value of preoperative Ca 19-9, in conjunction with dissected peripancreatic tissue margin, and confirmed that at higher preCA19-9 corresponds a worse prognosis.

Moreover, several Authors investigate which prognostic value, if the pre- or post-operative

serum CA19-9 level, is more useful in predicting survival. Besides correlating preCA 19-9 levels with stage of disease, Ferrone and coworkers [55], showed that both a postoperative decrease in CA19-9 and a postoperative CA19-9 value of less than 200 U/mL are strong independent predictors of survival. In analyzing data of pre- and postoperative serum CA19-9 levels from 109 patients who underwent surgical resection for pancreatic cancer, Kondo et al. [56] considered significant the differences in overall survival between groups divided on the basis of four postoperative CA19-9 cutoff values (37, 100, 200, and 500 U/ml) but not significant those between groups divided on the basis of the same four preoperative CA19-9 cutoff values. They conclude that postoperative CA19-9 level is a better prognostic factor than preoperative CA19-9 level. All together, results from these studies suggest that: (i) preoperative CA 19-9 correlates with stage of disease; (ii) a median of pre operative CA 19-9 serum level <100 U/ml correlates with resectability (41–80 %) whereas levels >100 U/ml suggest advanced or metastatic pancreatic cancer (60–85 %) [30, 57]; (iii) postoperative normalization or a downward trend of the CA 19-9 serum level is associated with prolonged survival whereas elevated or failure of the CA 19-9 to decrease following pancreatic resection reflects residual disease or occult metastasis and portends a poor survival [58].

15.5.4 CA 19-9 Serum Levels as a Biomarker for Chemotherapy Response in Pancreatic Cancer Patients

Several studies have been performed investigating the utility of CA 19-9 for assessing the efficacy of chemotherapy for advanced pancreatic cancer. Willett et al. [59] measured serum CA 19-9 levels in 42 individuals before and following chemotherapy treatment with 5-flourouracil and irradiation, to define the potential role of this tumor marker in preoperative management of these patients. In comparing these CA 19-9 values with findings of restaging computed tomography (CT) scan and laparotomy, the Authors showed a correlation, statistically significant ($P=0.009$), between increased or decreased CA 19-9 levels and disease progression. Results suggest that monitoring of CA 19-9 appears useful for the identification of patients who manifest progressive tumor growth and metastasis in spite of this treatment. In analyzing data of CA 19-9 levels in 36 subjects receiving gemcitabine treatment, Halm et al. [60] demonstrated the utility of serial measurements of this marker, to evaluate the response to chemotherapy. Authors showed that patients with a decrease of CA 19-9 >20 % after 8 weeks of treatment ($n=25$) have a significantly better median survival compared to patients with a rise or a decrease < or = 20 % ($n=11$) $P<0.001$. Other more recent studies analyzing prospective trials showed similar results, suggesting that CA 19-9 is a prognostic and predictive biomarker in patients with advanced pancreatic cancer who receive gemcitabine-containing chemotherapy [61–63]. Moreover, on the basis of data, from 1997 to 2002, of 96 patients who underwent pancreatectomy without adjuvant chemotherapy as the control arm of a large randomized prospective adjuvant therapy trial, Hernandez et al. [64] concluded that CA 19-9 velocity predicts disease-free survival and overall survival after pancreatectomy of curative intent. According to previous results, Reni et al. [65], plotting the survival curves on a pre-defined decline in CA 19-9 serum levels of 247 advanced pancreatic cancer patients enrolled in five consecutive chemotherapy trials, illustrated that a higher percent decline in CA 19-9 serum levels following treatment corresponds to an improved overall survival. In spite of all these evidences, however, the NACB Panel recommends that serial CA 19-9 measurements during palliative chemotherapy should be used in conjunction with imaging tests to determine the efficacy of treatment. Serial CA19-9 monitoring is also recommended in the follow-up of patients after potentially curative surgery. Moreover, according to 2006 ASCO update of recommendations for the use of tumor markers in gastrointestinal cancer, CA 19-9 should not be used to define disease recurrence if not with the support of accurate evaluative imaging techniques.

15.6 Other Serum Markers for Pancreatic Cancer

Early detection of pancreatic cancer is an ever prominent problem, considering the high death rate for this disease. A wide range of potential new markers, including serum, pancreatic juice and tissue-based markers, have been proposed for early detection, as reported by the European Group on Tumor Markers (EGTM) [1]. Among these, duke pancreatic monoclonal antigentype 2 (DUPAN-2), macrophage inhibitory cytokine (MIC-1) and regenerating islet derived (REG-4), being unaffected by Lewis blood group status, may be more effective for detecting the presence of pancreatic cancer in sialyl Lewis negative population [66]. Additional tissue-based markers have been object of a series of studies, reporting initially promising results. For example, among possible oncogene/oncosuppressor mutations, which occur at various stages during proceeding of neoplastic disease, the most important are: K-ras, EGF e EFGR (precocious), of p16 and p53 (intermediate in the neoplastic evolution), and of SMAD and BRCA2 (more tardy). The KRAS is an oncogene that encodes a small GTPase transductor protein called p21, which participates in intracellular signal transduction and is involved in the regulation of cell division. Activating mutations in the KRAS gene impair the ability of the KRAS protein to switch between active and inactive states, inducing the active state. The resulting aberrant forms of p21 have a profound effect on the downstream effector pathways, resulting in much higher proliferation rates, enhanced cell survival and resistance to apoptosis that may evolve toward neoplastic process. K-ras mutations are frequently observed in human cancers [67] and are reported to be present in about 90 % of pancreatic ductal carcinomas, appearing in the relatively early stages of carcinogenesis [68]. The mutations found most frequently in the KRAS gene of cancer cells are located at positions 12 and 13 in exon 1, and less frequently in codons 61, 63, 117, 119, and 146 [69]. In particular, mutations in codons 12 or 13, which are present with high frequency in pancreatic cancer, are known to lead to conformational changes in the KRAS protein. The majority studies analyzing the potential biomarker role of KRAS mutations in pancreatic adenocarcinoma show those mutations as an adverse prognostic indicator, others, however, does not found significant relationship between the presence of mutant K-ras and poor outcome. The resulting data, obtained moreover by using methods with varying sensitivities and specificities to determine K-ras mutant, are still conflicting, as reported in a systematic review of the literature of Garcea et al. [68]. The available evidence do not sustain till now the use of K-ras for routinely determining prognosis in patients with pancreatic cancer. Of similar limitations suffer studies that related p53 mutation/overexpression to outcome in patients with pancreatic cancer [68]. As well known, p53 tumor suppressor gene encodes a transcription factor which is involved in regulating cell cycle, apoptosis, and has been defined “the guardian of genome” [70], because of its role in conserving stability by preventing genome mutation [71]. Mutations in the p53 gene are frequently found in human cancer, and are present in a percentage ranging from 50 % to 70 % of pancreatic cancers, appearing relatively late in the genesis of this malignancy. However, available conflicting data does not permit to establish a strict association between p53 status and patient outcome.

Also mucins are extensively studied in relationship with pancreatic cancer [72]. As well known, mucins are high molecular weight glycoproteins widely expressed by specialized epithelial cells of the gastrointestinal, respiratory, and urinogenital tracts. Under normal circumstances, mucins are known to play a protective role for epithelial tissues. However, in numerous pathologic situations, their aberrant expression is known to have multiple implications in development, progression, metastasis and a poor prognosis of cancer [73,74]. In particular, MUC1, MUC2, MUC4 and MUC5AC are key mucins in pathological diagnosis of pancreatic neoplasm. In 2007, Wang and coworkers, in immunohistochemically confirming the aberrant expression as well as changed in the level and distribution pattern of mucins (MUC1, MUC2 and MUC5AC) in pancreatic cancer, furthermore observed that the

combined implementation of conventional imaging technique and molecular diagnostic approaches may provide improved sensitivity and specificity of diagnosis of pancreatic cancer and mucinous neoplasms. In particular, the Authors reported that the combination test of MUC1 + cytology and MUC5AC + cytology could improve sensitivity (respectively 85 % versus 65 %, 100 % versus 65 % of cytology alone) and accuracy (89 % versus 73 %, 91 % versus 72 % of cytology alone) for pancreatic cancer diagnosis. Also the combination test of MUC2+cytology and MUC5AC + cytology could achieve higher sensitivity (78 % versus 39 %, 100 % versus 39 % of cytology alone), specificity (97 % versus 60 %, 71 % versus 60 %) and accuracy for mucinous neoplasm diagnosis. Recently, Yokoyama et al. [75] showed that three mucin genes (MUC1, MUC2 and MUC4) expression in cancer cell line was regulated by DNA methylation and analyzed the DNA methylation status of mucin genes by a 'methylation-specific electrophoresis' method to high sensitivity and resolution. Results from pancreatic juice samples from 45 patients with various pancreatic lesions indicated that the DNA methylation status of MUC1, MUC2 and MUC4 in pancreatic juice with the mucin expression in tissue. Analyses of the DNA methylation status of MUC1, MUC2 and MUC4 of human pancreatic juice may provide useful information for differential diagnosis of human pancreatic neoplasms, with specificity and sensitivity of 87 % and 80 % for PDAC. In an attempt to define the cellular and molecular mechanisms through which MUC4 contributes to the metastasis of pancreatic cancer cells, Senapati et al. demonstrated that MUC4-NIDO domain interaction may play a role in promoting the breaching of basement membrane integrity and spreading of cancer cells [76].

More recently, the discovery of miRNA, small non-protein-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level, seems to open new ways not only in oncology research but also in cancer therapeutics. A growing number of direct and indirect evidence demonstrates that miRNAs expression is profoundly altered in human cancer or strongly modulated during carcinogenesis. Moreover, the peculiar features of miRNAs, including their tissue- and disease-specific expression and their high stability in tissue and fluids, together with the possibility to detect them in very low amount of samples, may provide important advantages for supporting the possible use of miRNA as diagnostic and prognostic/predictive biomarkers and therapeutic targets. Following the first report in 2007 by Lee and coworkers [77], who identified a differential miRNAs expression profile in clinical specimens of pancreatic adenocarcinoma and pancreatic cancer cell lines, Wang et al. [78], 2 years later, detected miRNA in the blood of patients with PC. They showed that plasma profiling of four miRNAs (miR-21, miR-210, miR-196a and miR-155) can differentiate cancer patients from healthy controls, revealing a sensitivity of 64 % and a specificity of 89 % for pancreatic cancer. Afterwards, a series of researches have been performed for characterizing the miRNAs expression profile, highlighting a clear discrimination between pancreatic cancer, chronic pancreatic and normal pancreas. From all these translational studies, a panel of miRNAs whose expression results profoundly altered in PC is emerging [79]. Interestingly, a series of miRNA which are either upregulated (e.g. miR-146) or silenced (e.g. miR-205 and miR-7) was recently identified in advanced pancreatic cancer clinical samples as well as in pure populations of CSCs isolated from pancreatic cancer cell line resistant to gemcitabine [80]. In their recently published miRNA analysis from plasma of 140 pancreatic cancer patients, Liu et al. [81] support the diagnostic utility of the combination of plasma miRNAs (miR-155, 181a, 181b and 196a) with serum CA19-9 for early detection of pancreatic cancer. Using logistic modeling analysis, they proved that major effectiveness in combining miR-16, miR-196a and CA19-9 for discriminating PCa from non-PCa (normal + CP) (AUC-ROC, 0.979; sensitivity, 92.0 %; specificity, 95.6 %), and for discriminating PCa from CP (AUC-ROC, 0.956; sensitivity, 88.4 %; specificity, 96.3 %) compared with the miRNA panel (miR-16 + miR-196a) or CA19-9 alone. Importantly, the combination was

reported to be effective at identification of tumors in Stage 1 (85.2 %). Similarly, Wang et al. [82] identifying in peripheral blood mononuclear cells (PBMC) specific microRNAs whose levels might facilitate diagnosis of pancreatic cancer, evaluated their predictive value by logistic regression models, showing that a combination of PBMC miR-27a-3p and serum CA19-9 levels provided a higher diagnostic accuracy with a sensitivity of 85.3 % and specificity of 81.6 % (AUC = 0.886; 95 % CI, 0.837–0.923 %). Last, to assess the diagnostic value of the serum miRNA profiling, Liu et al. [83] identified a panel of seven miRNA (miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191), which appear to have an high sensitivity and specificity for distinguishing various stages of PaC from cancer-free controls and to accurately discriminate PaC patients from chronic pancreatitis patients. The diagnostic accuracy rate of the 7-miRNA profile was 83.6 % in correctly classifying 55 cases with clinically suspected PaC.

All these evidences suggest that miRNAs profiling may be used as potential tool for the early stage PC diagnosis, monitoring cancer progression and efficacy of the treatment. Another interesting aspect that is attracting the attention of oncologic research is the therapeutic potential of miRNAs. Recent studies demonstrate that microRNAs may soon translate into clinical applications not only as screening tools but also as therapeutic targets for this cancer. In fact, the possibility to modulate the miRNAs expression, by activating tumor suppressive miRNAs and by inhibiting oncogenic miRNAs with small molecules or gene transfer, seems to open new ways for the development of cancer therapeutics. This potential therapeutic aspect is very intriguing. At present, however, this application remains still a challenge and requires further in depth studies.

15.7 Conclusions

A large number of putative biomarkers derived from serum, tissue, bile, pancreatic juice and saliva has been proposed and are currently undergoing evaluation for pancreatic cancer detection. At present, most of them lacks large scale validation and however none of them has showed to possess the sensitivity and specificity required to be employed individually in early detection of pancreatic cancer. Therefore, although with important limitations we well-know, ranging from false negative results in sialyl Lewis negative subjects to false positive results in the presence of obstructive jaundice, CA 19-9 continues being the only pancreatic cancer marker of actual clinical use. However, because of its low positive predictive value, serum CA 19-9 determination cannot be used as screening marker, while it can be used in aiding diagnosis, in conjunction with results from accurate radiological procedures, in symptomatic patients. Measuring preoperative serum CA19-9 level is useful for evaluating the resectability of pancreatic cancer and for predicting the disease course. The inverse correlation existing between CA 19-9 levels and median survival of patients renders serum CA 19-9 a good marker for estimating overall survival of the patient and for evaluating the possible presence of residual disease after pancreatic resection. Serial CA 19-9 monitoring can be useful in the follow-up of patients during chemotherapy for appraising the efficacy of treatment.

Poor prognosis of pancreatic cancer patients makes the research of new sensitive and specific markers necessary to identify this malignancy at early stages of development. The possibility of a timely therapeutic intervention should assure a more effective treatment and could translate in a real improvement in the patients' survival but also in their of quality of life during the course of the illness.

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