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Clinical Use of the Tumor Marker Tissue Polypeptide Specific Antigen in Gastrointestinal Cancer

Przydatność kliniczna swoistego polipeptydu tkankowego jako markera w raku żołądkowo-jelitowym

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Abstract

TPS (tissue polypeptide specific antigen) is a well-documented tumor marker for epithelial malignancies. It measures an antigenic determinant associated with human cytokeratin 18, utilizing an epitope defined by the monoclonal antibody M3. TPS is a marker of tumor cell activity, rather than the more commonly used markers related to tumor burden. The main clinical value of TPS lies in early detection of recurrent disease and in rapid assessment of treatment efficacy. Decreasing TPS levels during therapy monitoring indicate response and in case of a fast decrease, a favorable prognosis is often indicated. Further, increasing TPS levels in patients with clinically stable disease or partial remission is a reliable indicator of progression with a considerable lead time. When tumor marker determinations are applied in a proper way in the appropriate situation, the biomarker results can assist the oncologist. TPS has been shown to be of value in gastrointestinal malignancies, the site of more cancers than any other body organ system as exemplified by colorectal, gastric and pancreatic cancers. In advanced colorectal cancer, TPS is a powerful parameter during post-surgical follow-up and in monitoring palliative treatment. TPS levels significantly correlate with clinical outcome and therapy response. TPS is associated with aggressive disease and provide also prognostic information in colorectal cancer. In pancreatic cancer, TPS is a valuable indicator of the malignant process, important as a highly discriminative marker for differential diagnosis of pancreatic carcinoma and chronic pancreatitis. Increased TPS levels are a powerful parameter that supports the identification and differentiation of the cancer patients with high sensitivity and specificity (Adv Clin Exp Med 2006, 15, 1, 11–16).

Key words: TPS, cytokeratin 18, gastrointestinal cancer, colorectal, pancreas, diagnosis, prognosis, monitoring.

The Cytokeratin Family

The cytoskeleton is composed of three types of filamentous structures: microfilaments intermediate filaments and microtubules [1]. The intermediate filament is the most complex group with respect to protein composition (cytokeratins, vimentin, desmin, glial filaments and neurofilaments) [2, 3]. Cytokeratins are found in epithelial cells and form an intracellular network of filamentous structures, whose major function is to provide the physical integrity and architecture of the cell and also the position of the organelles. Twenty different cytokeratin proteins are known at present. Based upon biochemical data and gene sequence relationships, the cytokeratins can be grouped in two families: cytokeratins 1–8 constitute the type II group (53–68 kDa, neutral to basic protein components); while cytokeratins 9–20 constitute the type I group (40–56 kDa, acidic proteins) [2, 4, 5]. Cytokeratin proteins are expressed as obligate heterodimers, containing a type I and a type II protein, which form tight interactions and are organized in stoichiometric amounts. This is exemplified by the combination of the cytokeratins 8 and 18 [6]. The heterodimers are further assembled into filamentous structures by aligning side-by-side to form tetramers and end-to-end to form coiled-coil higher polymeric structures [1, 3, 7, 8].
Expression of Cytokeratins

The expression of cytokeratins varies with epithelial cell type, the level of differentiation and the extent of development of the tissue. Stratified squamous epithelia express mostly cytokeratins 1–6 and 9–17, while cytokeratins 7, 8 and 18–20 are identified in simple epithelia. Of the latter, cytokeratins 8, 18 and 19 are the most abundant ones in malignancy [6].

A few biological characteristics are noteworthy, making cytokeratin proteins valuable targets as tumor markers: the cytokeratin expression pattern is usually maintained during the transformation of normal cells into malignant ones [9, 10]; the cytokeratins demonstrate very low solubility in the cytoskeleton, but when present in the circulation cytokeratins are easily detected as partially degraded protein fragments, in smaller or larger complexes [11]; the half-life of the soluble fragments in the circulation, depending on fragment size, is about 10–15 hours. Intact non-degraded cytokeratin molecules have not been demonstrated in the circulation [11].

Multiple pathways appear to be involved in the processes leading to the release of soluble cytokeratin fragments into the circulation. Although not yet completely known, proteolytic degradation in dying cells, abnormal mitosis, leakage from rapidly proliferating cells, apoptosis and neovascularization are all likely to play important parts. All these processes are to a large extent connected with the malignant processes. In apparently healthy individuals the cytokeratin levels in the circulation generally is low, but rises significantly and rapidly in patients with epithelial cell associated carcinomas. A few benign conditions can also give rise to increased levels, although not to the same extent.

The soluble cytokeratin fragments can be detected in a number of body fluids, like blood, urine, ascites, pleural effusions, cerebrospinal and cyst fluids.

Apoptosis and Cytokeratins

Apoptosis, the sequence of events leading to controlled cell death, is a central biological process in the overall control of cell function [12]. The level of this cellular pathway’s importance cannot be underscored and is directly appreciated when studying its deregulation, where the failure of cells to undergo apoptotic cell death is involved in a variety of human diseases. For the development of cancer, a deregulated apoptotic pathway is a key event.

Although the exact role is unclear, cytokeratin proteins have become of interest lately for their involvement during apoptosis [13, 14]. The morphological features of apoptosis such as cell shrinkage, nuclear fragmentation and apoptotic body formation arise from caspase cleavage of specific cellular substrates. Likely, type I cytokeratins are substrates for caspases, having specific and conserved recognition motifs, while type II cytokeratins do not appear to undergo caspase mediated digestion [5, 8, 14, 15].

It has been suggested that caspase cleavage of the cytokeratin proteins facilitates the formation of the apoptotic bodies and amplifies the apoptotic signal [2, 14]. Extracellular release of two type I cytokeratins, 18 and 19, has in vitro been shown to be a direct consequence of caspase digestion during the intermediate apoptotic stage, thereby serving as markers of apoptosis [16].

Quantification of apoptotic epithelial cells at the level of the cytoskeleton shows a correlation with known molecular properties of caspase cleavage sites, as demonstrated by the recently introduced M30-ApoptoSense assay measuring caspase cleaved fragments of cytokeratin 18 in the supernatant of epithelial apoptotic cell cultures [17]. This provides a molecular basis to the early apoptotic event and a possibility to follow therapeutic interventions when apoptotic processes are out of balance.

Cytokeratins as Tumor Markers

Cytokeratin markers reflect tumor cell activity. Their ability to predict disease status prior to conventional biomarkers and traditional diagnostic methods is well known. They have a well established role in the management of patients with epithelial cell carcinomas, particularly in monitoring and follow-up, where they offer reliable and cost efficient tools. Cytokeratin markers react fast on changes in tumor growth by distinct increases or decreases in marker levels. However, like other commercially available tumor markers measuring tumor volume, cytokeratins are not “organ specific”.

There is an increasing interest to use combinations of tumor markers, clinically established tumor marker panels, instead of single markers. The advantage is an increased sensitivity, leading to a more complete clinical picture of the individual patient. The combination of a cytokeratin activity marker with a marker reflecting the tumor burden during therapy monitoring and in follow-up has been shown to be advantageous.

Few cytokeratin markers are commercially available. The most widely applied markers are
TPA (tissue polypeptide antigen), CYFRA 21-1, and TPS (tissue polypeptide specific antigen). TPA is a broad-spectrum assay that measures cytokeratins 8, 18 and 19; while CYFRA 21-1 and TPS are more specific and measure cytokeratin 19 and 18, respectively. These assays have been applied in many different types of epithelial cell carcinomas. Although based on detection of the same type of proteins in serum, the individual cytokeratin immunoassays may give different profiles of reactivity reflecting the differences among the individual cytokeratin assays. Cytokeratin tumor markers, as other assays, are not interchangeable, and therefore it is important to define which cytokeratin marker should be applied in the specific clinical setting [18].

This review is focused on the clinical use of TPS in gastrointestinal disease, with examples from the management of patients with colorectal and pancreatic diseases.

**TPS – a Cytokeratin 18 Marker**

Monoclonal antibodies were raised against antigenic structures of cytokeratin 18 in mice with human carcinoma preparations. The monoclonal antibody, M3, which reacts with one of two antigenic determinants on cytokeratin 18, was used to develop a TPS immunoassay for quantitative measurements [19]. N-terminal sequence analysis and cDNA gene cloning were applied to characterize the epitope structure recognized by the M3 antibody. The M3 binding region is located in the helical coil 2 domain of human cytokeratin 18 [11]. The antigenic structure recognized by TPS is mostly cytokeratin 18 fragments and complexes expressed in malignant epithelial tissues.

TPS is a serum biomarker measuring soluble fragments of cytokeratin 18 in the circulation and is consequently able to contribute in predicting prognosis, monitoring treatment and response to treatment as well as prediction of recurrence in different carcinomas [18]. Biological fluctuations can be observed in the serum levels of cytokeratin markers including TPS. Further, elevated levels can be anticipated for various benign conditions, like liver disease, renal failure, diabetes and general infections. Commonly these conditions are already known, therefore tumor marker levels in these cases should be interpreted with caution.

TPS is a well documented marker in various epithelial cell-associated carcinomas [20–29], with approximately 400 scientific publications covering different aspects of the serum tumor marker, from basic research to applications in clinical oncology.**

**TPS in Clinical Use**

TPS has been examined in many different types of epithelial carcinomas, as single marker or in combination with markers reflecting the tumor burden. Its clinical impact in patient management, therapy monitoring and prediction of recurrence is well known for a number of cancer diseases, in particular breast, prostate, ovarian and gastrointestinal cancers.

**Colorectal Cancer**

Biochemical markers for colorectal cancer are potentially useful for diagnosis of early disease, determining prognosis, predicting response to specific therapy, surveillance of patients undergoing curative resection and monitoring treatment of advanced disease.

CEA has been the marker of choice in colorectal cancer, for prognosis and as an early indicator of recurrence in the follow-up after primary surgery. It is well known that the sensitivity of CEA in primary disease is not sufficient and that the addition of another serum marker (multiple markers) might provide greater sensitivity at acceptable specificity.

Cytokeratin markers and in particular TPS (measuring tumor cell activity in colorectal cancer) have been shown to be of benefit in follow-up of colorectal cancer patients to detect the onset of metastatic disease, and for preoperative prognosis in Duke’s D patients with disseminated disease, where patients with lower tumor marker levels showed significantly longer overall survival compared to patients with elevated marker levels [30–32]. Multivariate analysis could show that staging, and the tumor markers CA 242 and TPS were the strongest prognostic factors in decreasing order, while TPA was not an independent prognostic factor [31]. The latter further emphasizing the different reactivity patterns among the cytokeratin assays.

Chemotherapy is widely used for palliation in patients with advanced colorectal carcinoma. The aim of the treatment is to decrease tumor-related symptoms, reach an increased symptom-free period and to prolong survival. In this setting, the reliability and validity of the serum markers TPS, CEA, VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) were evaluated to monitor palliative chemotherapy in 87 patients with advanced colorectal cancer [29]. These patients were treated with a combination first-line treatment: 5-fluorouracil and leucovorin before and 2, 4 and 10 weeks after induction. The
eight patients with normal baseline TPS levels subsequently showed a favorable outcome with prolonged survival and a higher rate of objective response than patients with elevated TPS levels. At 10 weeks, all responders had a TPS decrease of > 25% and this correlated significantly, even in multivariate analysis, with both objective and subjective responses. The sensitivity for this decrease was 83 and 86% for objective and subjective response, respectively (specificity 65 and 72%, respectively). CEA in the same setting showed 45% and 46% objective and subjective sensitivity respectively, and a specificity of 88%. VEGF and bFGF were elevated in 54 and 15% of the patients respectively. Changes in VEGF, even when decreasing during therapy, did not show any correlation to survival or response. Combined use of the tumor markers did not enhance the predictive value compared to TPS alone [29].

Repeated measurement of TPS levels during therapy can be of clinical relevance, primarily as a marker of lack of response. In this context, TPS is clearly superior to the more commonly used CEA. In many asymptomatic patients that still desire active treatment because of their generally poor prognosis, changes in elevated TPS levels appear to be of value in guiding the length of treatment [28, 29].

In another study on monitoring of palliative chemotherapy, TPS was analyzed in 90 patients with various advanced gastrointestinal cancers, as divided by 52 patients with colorectal cancer, 9 pancreatic cancer, 11 biliary cancer, and 18 patients with gastric cancer. Serum was taken prior to every treatment course to explore whether serial measurements could be of importance in monitoring patients on palliative chemotherapy [28]. Elevated TPS levels were seen in 92% of the patients (83/90 patients overall; 48/52 colorectal, 9/9 pancreatic, 9/11 biliary, 17/18 gastric). Baseline TPS level correlated with performance status, tumor response and survival. Changes in TPS levels after the first two courses in relation to baseline value (> 50% decrease) showed a high sensitivity for a favorable treatment outcome: partial remission/prolonged stationary disease (90%) or subjective response (100%). A similar result was seen when analysis of TPS levels at the time of response evaluation after 2 months gave 91% and 95% sensitivity. In 7/15 with initially favorable outcome, increased TPS levels (> 50%) at two occasions was seen with 8–20 weeks lead time prior to clinically identifiable progression. The authors concluded that serial TPS measurements in patients with advanced gastrointestinal cancer, with high accuracy early can identify patients who would not benefit from the treatment. TPS measurements before and during palliative chemotherapy may save toxicity for the individual patient and reduce costs for society [28].

**Pancreatic Cancer**

Methods for early detection of adenocarcinoma of exocrine pancreas are urgently needed. Studies on individuals on their first visit have been carried out using the serum marker CA 19-9, which has been widely used for the diagnosis of pancreatic cancer. To improve the effectiveness of the diagnosis for pancreatic cancer it is necessary to use more cancer-specific tumor markers or combination of markers.

Distinguishing between malignant pancreatic carcinoma and chronic pancreatitis is still difficult despite significant improvements in diagnostic imaging techniques. CA19-9 is currently the most widely used biomarker for pancreatic carcinoma when used in combination with accepted diagnostic procedures in patients diagnosed and treated for pancreatic cancer. The application of CA19-9 separate in diagnostic procedures is not satisfactory because it lacks sensitivity and specificity. Elevated circulating levels of CA 19-9 can be found in patients with benign pancreatic diseases, as well as in patients with other gastrointestinal malignancies [33].

In a recent study the ability of the tumor markers TPS and CA 19-9 to discriminate between malignant pancreatic tumors and chronic pancreatitis was analyzed [34]. One hundred twenty-two patients suspected of having either pancreatic carcinoma or chronic pancreatitis were enrolled in the study. Forty-eight patients presented with pancreatic carcinoma, of which two were excluded from further analysis. The TPS level was elevated (cut-off 100 U/l) in 100% (46/46) of the carcinoma patients. Elevated CA 19-9 levels (cut-off 37 U/ml) was seen in 32/46 (70%) of the patients. Chronic pancreatitis was recognized in 74 patients and in these patients elevated TPS and CA 19-9 levels were seen in 22% and 19%, respectively. Median levels of both markers were significantly higher in patients with pancreatic carcinoma than chronic pancreatitis. By introducing a higher cut-off value for TPS, 200 U/l, most of the false positive results for chronic pancreatitis could be eliminated (slightly reduced sensitivity, 97%, while the specificity increased to 98%). Increasing the cut-off point for CA 19-9 did not improve the differential diagnosis capacity. The authors concluded that TPS was a powerful indicator of the malignant process in the human pancreas, and that it, when compared to CA 19-9, had a significantly higher sensitivity and specificity for differentiating between pancreatic carcinoma and chronic pancreatitis. It would
appear that testing for TPS in patients with chronic pancreatitis is helpful in monitoring the proliferative process. The use of a higher cut-off value for TPS has considerable merit [34]. Some of the patients were further followed in a long-term clinical follow-up study. A total of 15 of the patients with pancreatic carcinoma (out of 46) and 11 of the patients with chronic pancreatitis (out of 74) could be included. Median follow-up time was 12.4 months (6.7–31.8 months) for the patients with chronic pancreatitis, and 20.7 months (3.1–29.8 months) for the patients with pancreatic cancer. In all patients with chronic pancreatitis (11/11) the initial TPS marker level was below the applied differentiating cut-off point of 200 U/l as established previously, whereas CA 19-9 was elevated in 2/11 patients. In one patient, a dramatic TPS increase (820 U/l) was measured at the last follow-up visit (after 8.6 months), which led to detection of a pancreatic cancer [35].

In all patients with pancreatic cancer the pre-operative TPS level exceeded 200 U/l, whereas CA 19-9 was elevated in only 9/15 patients. After surgery, 11/15 patients showed no evidence of disease; 8 patients with both markers within reference range and three patients with increased CA 19-9. No false positive cases were observed. In the remaining 4/15 patients, both TPS and CA 19-9 were clearly elevated and later on all these patients were diagnosed with distant metastases after the last marker measurement. The authors conclude that TPS reflects the clinical status of patients more accurately than CA 19-9 during long-term follow-up of patients with chronic pancreatitis and pancreatic cancer. In the group of patients with chronic pancreatitis, a marked increase in the TPS concentration indicated disease progression earlier than clinical recognition of cancer, and in the group of 11 patients with no evidence of disease, stable TPS levels correctly reflected the clinical situation [35].

A possibility of having a sensitive tumor marker for serial monitoring of patients with chronic pancreatitis would be of great benefit since rigorous clinical surveillance including diagnostic imaging procedures is difficult. Here, the clinical impact of TPS as a management tool is obvious, since increased levels supported the diagnosis of pancreatic cancer in patients with chronic disease.

Main Features for Clinical Use

As mentioned earlier, the clinical features using TPS in management of patients with epithelial cell carcinomas has been well demonstrated in numerous publications. This short review has focused on a few selected papers on TPS in gastrointestinal cancer, and can be summarized as follows: In advanced colorectal cancer, TPS is a powerful parameter during post-surgical follow-up and in monitoring palliative treatment. TPS levels significantly correlate to clinical outcome and therapy response (85% sensitivity). Furthermore, serial TPS determinations are of clinical relevance also as an early marker for prediction of the lack of therapy response in colorectal cancer patients and constitute an important complement to CEA for patient management [28, 29]. TPS is associated with aggressive disease and provide also prognostic information in colorectal cancer patients.

In pancreatic cancer, TPS is a valuable indicator of the malignant process, important as a highly discriminative marker for differential diagnosis of pancreatic carcinoma and chronic pancreatitis. Increased TPS levels are a powerful parameter that supports the efficient identification and differentiation of the cancer patients with 97% sensitivity at 98% specificity. On the contrary, the contribution from the traditional serum marker CA 19-9, as discriminant in pancreatic disease, was very low [34, 35].

References


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