TPS™ A CIRCULATING TUMOR MARKER IN BREAST CANCER

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ABSTRACT

Tissue polypeptide specific antigen (TPS) measures an antigenic determinant associated with human cytokeratin 18. TPS is a marker of tumor cell activity in contrast to markers related to tumor burden. The value of detecting circulating TPS lies in the early detection of recurrence by serial determinations and in the rapid assessment of the efficacy of the treatment. Pretreatment levels of TPS in patients with metastatic breast cancer are related with prognosis. Decreasing TPS levels during therapy monitoring indicate response and a fast response is correlated to favourable prognosis. Increasing TPS levels, in the presence of clinically stable disease or partial remission, predict disease progression with a considerable lead-time. Improved effectiveness in breast cancer management can be seen when TPS is used in combination with CA 15-3. When tumor marker determinations are applied in a proper way in the appropriate situation, the results can assist the oncologist. Thus monitoring of therapy in patients with metastatic breast cancer should be based upon serial TPS and CA 15-3 determinations in serum. The use of tumor marker determinations in the early follow-up interval following surgery to detect early tumor recurrence may be simpler, more sensitive and less expensive than imaging methods.

KEY WORDS : Tumor markers, TPS, Breast cancer management

INTRODUCTION

The cytoskeleton is composed of three types of filamentous structures; microfilaments, intermediate filaments and microtubules. The intermediate filament is, by far, the most complex group with respect to protein composition (cytokeratins, vimentin, desmin, glial filaments and neurofilaments). Cytokeratins found in epithelial cells, 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 into two families: cytokeratins 1-8 constitute the type II group (53-68 kDa, neutral to basic proteins) and cytokeratins 9-20 constitute the type I group (40-56 kDa, acidic proteins). The cytokeratins pair into obligate heterodimers containing one cytokeratin of type I and one of type II, which form tight interactions and are organized into filamentous structures by aligning side-by-side to form tetramers and end-to-end to form coiled-coil structures (1,2).

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The expression of cytokeratins varies with cell type, the extent of differentiation, and the development of the tissue. During the transformation of normal cells into malignant cells, the cytokeratin patterns are usually maintained. This property has enabled cytokeratins to be used as tumor markers (3). Cytokeratins 8, 18 and 19 are the most abundant cytokeratin proteins in carcinomas. They are released from tumor cells and can be found in the blood, cyst fluids, ascites and urine. Cytokeratins are present in the circulation as partially degraded single protein fragments in small complexes or as large polymeric protein complexes (4).

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 (related to cancer disease), was used to develop a TPS immunoassay for quantitative measurements. N-terminal sequence analysis and cDNA gene cloning were applied to characterize the antigen structure recognized by the M3 antibody (4). These studies revealed that the M3 binding region is located in the helical domain (coil 2) of human cytokeratin 18. The antigenic structure assayed by TPS (cytokeratin 18 fragments and complexes) is expressed in malignant epithelial tissues. TPS is a serum marker measuring soluble fragments of cytokeratin 18 in the circulation and is thus able to contribute in predicting prognosis and response to treatment in different carcinomas (5-10). Several markers have been studied in different types of epithelial cell associated cancer. However, it is important to define which markers

contribute to patient management. The most widely used markers for management of breast cancer patients are the mucin associated cancer antigens (eg. CA 15-3, CA 549, MCA) and CEA. These markers have mostly been used in indicating the risk, presence, tumor status and future outcome of cancer and therapy monitoring. CA 15-3 is the established marker in breast cancer, but there are disadvantages of using CA 15-3 alone in monitoring breast cancer patients post-operatively; low incidence of CA 15-3 detection in early stage disease (micrometastatic disease) and further 25-30% of patients with clinically documented recurrence do not show elevated CA 15-3 levels (11). Cytokeratin 18 is overexpressed in breast cancer epithelium, which makes TPS measurement especially useful in breast cancer patients. TPS serum level is not simply a reflection of tumor mass, but rather tumor cell activity and thus the combination of CA 15-3 and TPS might open up new possibilities.

TPS in primary untreated breast cancer

Breast cancer remains one of the main causes of death in women of Western countries. Fifty percent of patients diagnosed with breast cancer without metastases relapse within 5 years after the initial diagnosis. Once recurrence has been identified, the possibilities of being cured from the disease are limited. A great number of parameters has been proposed for diagnosis of breast cancer including tumor markers; different mucins of which CA 15-3 is the best known, CEA and cytokeratins (e.g. TPS). CA 15-3 and CEA are the most commonly used tumor markers in breast cancer.

The use of several mucin markers simultaneously do not improve the efficacy obtained by using only one of them. For this reason, one mucin marker combined with CEA or TPS has been applied for the diagnosis of breast cancer. The sensitivity of mucin markers or CEA in patients with breast cancer is low (20-35%) and these markers are generally not useful for diagnostic purposes (12, 13). Tumor marker negativity in a patient with the suspicion of breast cancer does not exclude the presence of malignancy in primary breast cancer. The key prognostic factors in breast cancer are clinical stage, tumor size and nodal status. It has recently been reported that CA 15-3 sensitivity increased with stage (11), but this has not been confirmed in other studies (14). Of special note was the fact that serum levels of CA 15-3 and CEA in stage III disease were not considerably higher than in patients with early breast cancer (stages I and II) (14). The same controversy has also been reported for TPS, as only slightly higher levels were present with stage III patients compared with stage II patients (14). The sensitivity of TPS in primary breast cancer has been reported to be between 30-40% (14, 15), demonstrating the similar sensitivity as for CA 15-3 and CEA. These tumor markers are only of limited value in the diagnosis of primary breast cancer and none of the serum tumor markers studied proved to be a significant predictor of breast cancer (12-16).

TPS for early detection of recurrence

Since tumor markers are substances produced more by tumors than normal tissues, elevated concentrations can be detected in patients with metastases. It is logical that these

increases will gradually be a reflexion of tumor growth. When evaluating tumor markers to early diagnosis of recurrence, it is necessary to compare their sensitivity and specificity to imaging methods (X-ray, liver ultra sound, bone scintigraphy, etc). Serial tumor marker determinations are useful with breast cancer patients, who have shown no evidence of disease. CA 15-3 and CEA have been used in several follow-up studies, but they have not had sufficient sensitivity (11). They detected only 40-60% of patients with clinically confirmed recurrence. These findings might be correlated to the fact that these markers are often related to tumor burden. Additionally, these markers are not sensitive enough in the diagnosis of locoregional recurrence, where clinical examination is the method of choice (17). A new and more logical approach is to combine CA 15-3 with TPS, an activity marker measuring tumor cell activity, during the follow-up of breast cancer patients.

Klein et. al. (18) used TPS for identifying patients who developed metastases after surgery. TPS and CA 15-3 were studied in 93 breast cancer patients with no evidence of disease. Twenty patients developed recurrence during follow-up, 85% showed an elevated TPS signal, but only 64% of the patients showed pathological CA 15-3 levels. The major benefit of TPS in the management of breast cancer patients seemed to be as a complement to CA 15-3 to detect tumor recurrences, in whom CA 15-3 was not elevated. It was also reported (19), that TPS could identify 75% of the breast cancer patients (displaying no evidence of disease) showing recurrence and CA 15-3 identified 72%, while CEA could only detect 37%. The combined use

of TPS and CA 15-3 provided an easy and efficient method for detecting metastases during patient follow-up.

During therapy monitoring of 267 breast cancer patients after surgery, Giai et. al. (15) applied TPS and CA 15-3 as markers for detection of recurrence. Elevated levels of TPS was found in 85% (17/20) of the patients and 50% (10/20) for CA 15-3 during a follow-up time of three years. In this prospective study, TPS alone was more sensitive than CA 15-3 to detect recurrence. Tumor markers are useful tools in the early diagnosis of metastases as compared to other diagnostic methods (image analysis). However, it is still not accepted to initiate or change treatment based solely upon increasing tumor marker levels (treatment failures).

TPS in metastatic breast cancer

The development of metastatic breast cancer creates a situation where only palliative treatment of the patients are possible. CEA and CA 15-3 have been the marker of choice during the last ten years to monitor patients with metastatic breast cancer (13, 20). Several groups reported the advantage of using TPS in combination with CA 15-3 to monitor metastatic breast cancer (15,21-24). TPS was elevated more often than CA 15-3 in the case of bone metastases (23). After 3 months of treatment in patients with metastatic breast cancer, TPS responded in 68% of the patients as compared to CA 15-3 (49%) and CEA (38%) in patients with clinically confirmed no progression. Furthermore, the TPS response was earlier and faster than the markers measuring tumor burden. The correlation with clinical deterioration after 6

months of therapy was 44% for TPS, 33% for CEA and 28% for CA 15-3. The authors suggested that these results indicate that TPS was shown to be more sensitive and an earlier predictor of therapeutic response. Again TPS appeared to be especially sensitive in patients who presented with bone and soft tissue metastases as compared to patients with visceral metastases. In the study by Schuurman et. al. (22), it was found that the sensitivity of TPS was comparable with CA 15-3 at the time metastases developed (about 90%) and moreover that TPS appeared to show changes faster than CA 15-3. The authors suggested that the combined use of TPS and CA 15-3 is preferable in the monitoring of metastatic breast cancer patients.

Giai et. al. (15) also studied CA 15-3 and TPS in patients with distant metastases. In progressive disease, elevated levels of TPS and CA 15-3 were demonstrated in 85% and 50% of the patients, respectively. Increasing levels of TPS were independent of the site of metastases, whereas elevated levels of CA 15-3 were mainly related to visceral metastasis. The correlation of CA 15-3 and TPS with disease course (tumor response) in 60 patients with metastatic breast cancer was analysed by Pronk et. al. (25). The increase in serum levels of CA 15-3 and TPS (more than 25%) correlated significantly with the clinical response (treatment failure). The combination of the two markers is useful in the determination of response to treatment and is recommended to be used as an adjunct to conventional response evaluation.

Barak et. al. (24) studied metastatic breast cancer patients treated with Megace+ Interferon (ER positive patients) or with

Tamoxifen+Interferon (ER negative patients) using CEA, CA 15-3 and TPS. Lower levels of the markers were found in responder patients as opposed to non-responders; but only TPS levels decreased significantly in patients with stable disease. TPS was found to be the most sensitive marker and the best prognosticator with a lead time of 6.8 months compared to 3.9 months for CA 15-3 and 1.8 months for CEA. Survival analysis demonstrated a median survival time of 815 days for patients entering the study with low levels of TPS as opposed to a median survival time of 332 days for patients with high levels of TPS. Barak et. al. (26) showed that TPS provides important and accurate information in metastatic breast cancer patients regarding second-line Taxol treatment efficacy in 67 patients. Response to treatment was assessed by evaluation 50% decrease in the marker levels. TPS decreased in 60% of the patients, CA 15-3 in 41% and CEA in 29%. Median survival time of patients with low levels of TPS, CA 15-3 and CEA (pre-Taxol treatment) was 16.2, 13.3 and 16.2 months, respectively. In contrast, high levels of the markers (at the time for pre-Taxol treatment) showed a survival time of 9.2 months for TPS, 11.9 months for CA 15-3 and 11.2 months for CEA. Univariate and multivariate analysis of pretreatment marker levels were significant for TPS and CEA, but not for CA 15-3 in univariate analysis and only for TPS in multivariate analysis. It was concluded that TPS should be used in combination with CA 15-3 to identify patients, who might benefit from Taxol treatment.

In a large prospective study, including 129 patients with disseminated breast cancer the treatment response comparing UICC criteria and tumor marker changes of TPS, CA 15-3 and CEA

at 3 and 6 months after therapy was started (27, 28). Marker changes were analyzed for 50% decrease, the time to 50% decrease, 25% increase and the time to 25% increase (lead-time). At the start of the study, the elevated levels of the markers were 73% for CA 15-3, 69% for TPS and 59% for CEA. The combination of CA 15-3 and TPS demonstrated the highest sensitivity (87%). TPS showed a 50% decrease of the marker level in 69% of the patients (median time 1.1 months), CA 15-3 in 46% (median time 2.7 months) and CEA in 47% (median time 1.7 months). These results are based upon the number of patients with initially elevated marker levels and who showed a positive response of therapy (at least temporarily) according to UICC criteria. These marker changes preceded UICC criteria by several months. It was concluded, that decreasing marker levels indicate a positive effect of the applied therapy and should be continued even when clinically stable disease is indicated. Another interesting observation was that TPS increased more often than CA 15-3 or CEA. In an extended clinical follow-up, it was proven that increasing levels of TPS and CA 15-3, when clinically still partial remission or stable disease was present, indicated early development of progressive disease. In 30 patients out of 46, at least one of the markers had increased outside the reference range within the observation period of six months; TPS in 54%, CA 15-3 in 20%, CEA in 20%. The combination of TPS and CA 15-3 increased in 63% of the patients. All 30 of these patients subsequently developed progression of their disease. The prognostic sensitivity was 83%, 30% and 30% for TPS, CA 15-3 and CEA, respectively. The combination of TPS and CA 15-

3 increased in prognostic sensitivity of 96% for progressive disease. The median lead time was 8.0 months (range 2.1-16.8) for CA 15-3 and 7.0 months (range 1.5-29.0) for TPS. Therefore, the early increase of a marker, with clinically complete response, partial response or stable disease should not be taken as a false positive result, but as an early indication of relapse. This information gives possibilities for the clinician to alter patient management decisions. Another approach in metastatic breast cancer is in the

absence of readily measurable disease, where the combination of CA 15-3 and TPS levels can be used to document treatment failure.

The main indication of TPS in metastatic breast cancer is to monitor the activity of the disease and to measure the efficacy of therapy in patients with metastatic spread. The combined use of TPS and a marker measuring tumor burden increases the sensitivity of detection of recurrence.

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