

Editorial

Tumor biomarkers: PSA and beyond

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Laboratory medicine plays an increasingly important role in the management of cancer patients (1, 2). In none of the tumors has the advent of biomarkers changed the treatment in a more fundamental way than the introduction of prostate specific antigen (PSA) which has virtually transformed the management of prostate cancer. Prostate cancer represents a major public health problem. The last decade has witnessed a hitherto unprecedented progress in the treatment of prostate cancer, including surgical therapy, radiation therapy or systemic treatment. In the case of prostate cancer, laboratory medicine has contributed not only to an early diagnosis, but had an equally important impact on the management of the tumor during the entire course of the disease. In fact, the introduction of PSA has likely changed the course of the treatment of this tumor to the extent that we now distinguish between the ‘pre-PSA era’ and the ‘PSA era’. The assessment of the disease course and the effect of therapy in patients with prostate cancer are currently based largely on serial PSA measurements. In patients after the radical therapy of the primary tumor, detection of a sustained rise of PSA is the basis for the diagnosis of relapse, termed biochemical relapse, and leads to the institution of therapy even if there is no evidence of a tumor on the imaging studies.

Early diagnosis that results from the widespread use of PSA in the screening allowed more patients with localized tumors to be treated with local treatments, i.e., surgery or radiation therapy. Major progress has been achieved in the systemic treatment of prostate cancer. While metastatic prostate cancer still remains an incurable disease, anticancer therapies may prolong the patient survival, sometimes for several years. Hormonal therapy based on either surgical or medical androgen deprivation has been the mainstay of the treatment of metastatic prostate cancer for decades. Metastatic prostate cancer progressing despite castration levels of androgens, may respond to second-line hormonal therapies, however, these responses tend to be of short duration. Cytotoxic chemotherapy has been offered to patients with a tumor progressing despite hormonal therapy. In 2004 two large clinical trials with docetaxel have been published proving that chemotherapy actually prolongs survival in metastatic prostate cancer (3, 4). Duration of response to docetaxel is, again, limited. More recently, two new agents, carbazitaxel (5) and abiraterone (6), were shown to prolong survival in patients with metastatic castration-resistant prostate cancer progressing after docetaxel. Carbazitaxel is another cytotoxic agent

that in a randomized trial significantly prolonged survival in patients with metastatic castration-resistant prostate cancer failing docetaxel (5). Abiraterone is a hormonal agent that also prolonged survival in patients with metastatic castration-resistant prostate cancer after the failure of docetaxel (6). In addition, the administration of autologous dendritic cells has been shown to prolong survival in patients with metastatic castration-resistant prostate cancer (7). The introduction of these new therapies obviously results in new requirements for laboratory testing to assess the efficacy or to monitor the toxicity of the treatment.

PSA is a member of kallikrein and kallikrein-related peptidases (KLK) family of proteases that is the focus of a review in the present issue of *Clinical Chemistry and Laboratory Medicine (CCLM)* (8). *KLK* genes are clustered on the long arm of chromosome 19 and share exon/intron organization and similarity of protein sequences (8). *KLK* family comprises 15 homologous, single-chain proteases that are expressed by glandular epithelial cells and participate in a range of physiological and pathological processes, including semen liquefaction, skin desquamation, tooth maturation and also tumor progression (8). PSA (or *KLK3*) is currently the best characterized *KLK* family member and only *KLK* protease that is widely used as a biomarker. As outlined above, the utilization of PSA as a biomarker in the management of patients with prostate cancer represents a unique situation across the entire spectrum of malignant tumors. Circulating PSA is mostly complexed with α_1 -antichymotrypsin, α_2 -macroglobulin or α_1 -protease inhibitor (complexed PSA), while unbound PSA that is referred to as free PSA comprises of single-chain non-clipped forms called intact PSA. Differential information may be derived from measuring total PSA and free PSA as the elimination differs between complexed PSA and free PSA. Serial PSA measurement or correlation with other clinical parameters led to the introduction of concepts of PSA velocity, PSA density or PSA doubling time.

Importantly, the expression of PSA is under the control of androgen receptor (9). Thus, the changes of PSA concentrations in patients treated with hormonal therapy may be associated with the reduction of tumor mass with different kinetics compared to cytotoxic chemotherapy. Despite the fact that the decrease of PSA concentration is usually less dramatic after cytotoxic chemotherapy compared to hormonal treatment, the change of PSA concentration is an established surrogate endpoint in patients with castration-resistant prostate cancer treated with docetaxel (10). However, the daily clinical practice has taught us that the changes of serum PSA concentrations must be interpreted with caution as a phenomenon of

PSA surge denoting a rise of concentrations with subsequent decline has been described (11, 12). The duration of PSA surge is variable, and the rise of PSA concentrations may continue for up to 2 months, and this may result in a premature interruption of an effective treatment. Along with PSA, serum concentration of kallikrein-related peptidase 2 (KLK2), one of the other KLK proteases, represents another promising biomarker of prostate cancer (13–15).

The paper by Ulmert et al. in the present issue of *CCLM* examines the kinetics of PSA and KLK2 in prostate cancer patients treated with the gonadotropin-releasing hormone antagonist degarelix (16). Gonadotropin-releasing hormone agonists, such as goserelin have been used as an alternative to surgical castration in the treatment of prostate cancer for decades, but due to the agonist activity the utilization of these drugs could be accompanied by a flare phenomenon that may result in an increase of PSA or, in patients with metastatic prostate cancer, even in a transient progression of the disease symptoms. As the flare phenomenon is compounding the results of initial PSA measurement in patients treated with gonadotropin-releasing hormone agonist, the kinetics of PSA after medical castration may be examined only after gonadotropin-releasing hormone antagonists. An exponential decline of serum PSA and KLK2 concentrations was observed after degarelix administration. As expected, the decline of free PSA, intact PSA and KLK2 concentrations was significantly faster compared to complexed PSA and total PSA (16).

It has been established that uncomplexed prostate KLKs (i.e., free PSA, intact PSA and KLK2) perform better than total PSA in distinguishing benign and malignant lesions. In addition, the data presented by Ulmert et al. (16) also indicate that the uncomplexed prostate KLKs may also allow for an earlier estimation of response to hormonal therapy. The rapid estimation of the therapeutic response may be even more important in patients with castration-resistant prostate cancer treated with docetaxel or abiraterone. Moreover, dendritic cell-based immunotherapy prolongs survival in patients with castration-resistant prostate cancer, but has no apparent effect on the surrogate endpoints commonly used in clinical trials like response rate and progression-free survival (7). It remains to be determined by prospective studies whether serial measurements of uncomplexed prostate KLKs would provide a more rapid and reliable estimation of response to new therapies used in metastatic prostate cancer. Only clinical experience will determine whether the utilization of uncomplexed KLKs in the estimation of response to cytotoxic agents will eliminate some confounding phenomena, such as PSA surge.

Randomized clinical trials of prostate cancer screening have yielded conflicting results (17, 18). Overtreatment is a major problem in patients with early prostate cancer, resulting not only in unnecessary biopsies, but also in treatment being instituted in patients with indolent early prostate cancer, especially the elderly, who may be more threatened by the side effects of therapy rather than by the disease itself. It has been demonstrated that utilization of a panel of biomarkers that includes PSA forms and KLK2 could result in the reduction of biopsy rates (19).

Looking at the many issues still open with regard to the use of tumor biomarkers in prostate cancer, a disease in which the utilization of tumor biomarkers is firmly established, may lead to skepticism, nihilism or even desperation regarding the usefulness of biomarkers in most other tumors, where we may still lack biomarkers that would be helpful in the diagnosis or surveillance of patients. The research into more biomarkers may involve the identification of new molecules that could serve a tumor biomarker. In addition, while peripheral blood or tumor tissue currently represent the predominant sample matrices for biomarker determination, other sample matrices are being actively studied. In these 'alternative' sample matrices, biomarkers are encountered that are not present in peripheral blood or are present at higher concentrations compared to peripheral blood, or there is less 'background noise' caused by the presence of multiple other molecules.

The quest for the identification of new molecules as tumor biomarkers may be well illustrated on the example of KLK family. Apart from PSA and KLK2, the expression of other proteases of KLK family in tumor tissues, or concentrations of these molecules in biological fluids is being investigated for the potential use as tumor biomarkers (8, 13). For example, promising early results were reported for circulating concentrations of KLK6 in patients with epithelial ovarian cancer. Although two tumor biomarkers, carbohydrate antigen (CA) 125 and human epididymis protein 4 (HE4), are currently available and widely used in clinical practice in patients with epithelial ovarian cancer (20–22), an optimal biomarker (or, probably, a set of biomarkers) for early diagnosis of epithelial ovarian cancer and surveillance of patients already diagnosed with this tumor remains to be defined. Future studies may address the performance of KLK6 alone or in combination with CA125 and HE4 in patients with epithelial ovarian cancer. Unfortunately, the concentrations of virtually all circulating tumor markers that are being currently used in clinical practice could be increased as a result of a benign disorder (23), representing an important limitation for biomarker use in cancer diagnosis or detection or recurrence. This lack of specificity may sometimes be circumvented by the use of an alternative sample matrix, e.g., cerebrospinal fluid or malignant effusion, for biomarker determination.

As stated above either tumor tissue or peripheral blood (including plasma or serum) represent the sample matrices currently predominantly used for biomarker determination. However, other sample matrices, including cerebrospinal fluid (24, 25), urine (26–28), stool (29), secretions (30) or breath (31–33) have been explored in patients with benign or malignant conditions and, in cancer patients, may offer an advantage in certain situations over peripheral blood or tumor tissue. Malignant effusions, e.g., ascites (34–36) or pleural effusion (37), represent other interesting sample matrices that may allow a direct study of the changes associated with proliferation of cancer cells and host response against neoplasia in the tumor microenvironment.

Among potential alternative sample matrices, breath analysis has been one of the focal points of interest in recent years and volatile organic compounds present in the exhaled breath have been proposed as potential tumor biomarkers (31, 32, 38).

The utilization of exhaled breath as a sample matrix extends the concept of tumor markers that were originally defined as 'a special assay performed on a body tissue or fluid' (2). The topic of the exhaled breath analysis as cancer biomarker is reviewed in the current issue of *CCLM* in depth by Badjagbo (38). As a sample matrix, breath is far less complex compared to blood, tumor tissue or other biological fluids. Moreover, breath as a sample source is continuously available, and the sampling may be repeated without any restrictions. The biomarkers in breath are usually volatile organic compounds, including ethane, pentane, isoprene, benzene, toluene or xylene isomers. The volatile organic compounds are produced as a result of lipid peroxidation that is associated with cancer. Different techniques used to detect volatile organic compounds in the breath included gas chromatography combined with flame ionization detector, gas chromatography combined with mass spectrometry, proton transfer reaction mass spectrometry or selected ion flow tube mass spectrometry (38). Using these technologies, several promising biomarkers have been identified in the breath of cancer patients. For example, Buszewski et al. (31) analyzed volatile organic compounds released by non-small cell lung cancer (NSCLC) explants cultured in vitro and in the breath of NSCLC patients using solid phase micro-extraction and gas chromatography/mass spectrometry, and noted marked differences of volatile organic compound concentrations in the breath of patients and controls. The presence of tumor-specific biomarkers in the breath that may be detected by a very sensitive olfactory system of some animal species, e.g., dogs, also represents an explanation for the phenomenon of canine olfactory detection of cancer (39, 40). Although at first glance the topic of canine olfactory detection may seem to be from the realm of alternative medicine, this phenomenon is well documented as recently reviewed (39). For example, in a recently reported study the sensitivity of trained dogs to detect colorectal cancer when compared with colonoscopy was over 90% while the specificity for olfactory detection using stool and breath samples was 99% (41).

Most patients with metastatic castration-resistant prostate cancer are elderly and the risk of serious toxicity is high in this population. However, the treatment is effective only in some patients. There are patients in whom the therapy is not effective and who will experience serious toxicity. Biomarkers are urgently needed that would help with identifying the patients who are likely or unlikely to respond and patients in whom serious toxicity may be expected. One of the mechanisms responsible for the resistance of tumor cells to agents used in metastatic castration-resistant prostate cancer, e.g., taxanes, is the expression of P-glycoprotein (42), a membrane protein that mediates the efflux of xenobiotics, including cytotoxic agents. P-glycoprotein is one of adenosine triphosphate-binding cassette (ABC) transporters. The paper by Vaclavikova et al. on the prognostic significance of *ABCB1* gene polymorphisms in patients with breast cancer in the present issue of *CCLM* (43) brings new information of potential relevance in the prediction of response to cytotoxic agents. Single nucleotide polymorphisms of the *ABCB1* gene were associated not only with tumor size and phenotype, but also with the prognosis of

patients treated with adjuvant chemotherapy, indicating that genetic differences in P-glycoprotein function may determine the long-term outcome. Future studies to confirm these findings should also include evaluation in breast cancer patients treated with neoadjuvant chemotherapy. The major advantage of studying patients treated with neoadjuvant chemotherapy is the availability of an endpoint that reliably indicates the treatment efficacy and is strongly associated with the long-term outcome, i.e., the pathological response. Neoadjuvant chemotherapy is administered with the perspective of surgery being performed after chemotherapy administration. The thorough histological examination of the resection specimen reveals the response of the tumor to the treatment that can range from no visible changes to complete disappearance of tumor cells, termed pathological complete response. Although the study reported by Vaclavikova et al. (43) examined the relation of *ABCB1* gene polymorphisms with the response to neoadjuvant therapy, the number of patients examined was small and, most importantly, pathological response was not assessed. Pathological complete response rate differs dramatically based on breast cancer phenotype, and the significance of genetic polymorphisms should be studied in a population of patients with tumor phenotype associated with high pathological complete response rate, e.g., triple negative breast cancer.

Studies should also be performed in other tumors, including castration-resistant prostate cancer, and could provide important information regarding the selection of therapy for patients who are likely or unlikely to respond. Among the number of other factors responsible for the efficacy of cytotoxic agents in patients with solid tumors, the immune response may play an important role. For example, the presence of tumor-infiltrating lymphocytes has been associated with response to neoadjuvant chemotherapy in patients with breast cancer (44). More studies need to be done before patient selection based on genetic polymorphisms or parameters of immune response could enter clinical practice.

Biomarkers that are determined in the laboratory could also potentially play an extremely important role in detection and prediction of chemotherapy toxicity. For obvious reasons, the best established biomarker of treatment toxicity is peripheral blood cell count for hematological toxicity, but the utilization of laboratory methods in the assessment of toxicity spans different organ systems, and includes monitoring of gastrointestinal toxicity or cardiac toxicity of chemotherapy (45–47). The prediction of the risk of serious toxicity is even more important in the population of patients with castration-resistant prostate cancer. However, the potential of laboratory medicine in the assessment or prediction of side effects affecting other organ systems has so far not been fully translated into daily clinical practice.

In conclusion, the papers in the current issue of *CCLM* demonstrate the complexity of approaches in the development of cancer biomarkers. The utilization of PSA may serve as a paradigm for successful implementation of biomarker measurements into the management of different cancers. Potential future biomarkers may encompass a wide range of approaches, including breath analysis or determination of genetic polymorphisms.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

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