

Assessing the clinical significance of tumor markers in common neoplasms

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1. ABSTRACT

The term tumor markers include a spectrum of molecules and substances with widely divergent characteristics whose presence in the significant amount can be related to the malignant disease. An ideal tumor marker should have high specificity and sensitivity, which would allow its use in early diagnosis and prognosis of malignant disease, as well as in prediction of therapeutic response and follow-up of the patients. Numerous biochemical entities have emerged as potentially valuable tumor markers so far, but only few markers showed to be of considerable clinical reliability and have been accepted into standard clinical practice. Recent development of genomics and proteomics has enabled the examination of many new potential tumor markers. Scientific studies on discovery, development, and application of tumor markers have been proceeding quite rapidly providing great opportunities for

improving the management of cancer patients. This review is focusing on the clinical usefulness of various tumor markers already in clinical practice as well as certain potential markers, giving a brief description of their prognostic and predictive significance in most common malignancies.

2. INTRODUCTION

The term tumor markers, also called biomarkers, include a spectrum of molecules and substances with widely divergent characteristics that can be found in the body in significant amounts when cancer is present (1). Tumor markers include a broad range of biochemical entities such as cytoplasmatic proteins, enzymes, tissue receptors, antigens, oncogens, and hormones (2). Furthermore, tumor markers can also signify a process like apoptosis, angiogenesis, and proliferation that can cause

quantitative or qualitative intracellular alternations which are detectable by various assays (3). Alterations can be produced either by genesis and the growth of the tumor itself or by the surrounding normal tissue as a response to tumor cells or certain benign (non-cancerous) conditions (3). Alterations primarily occur in three main classes of genes including (proto)oncogenes, tumor suppressor genes and DNA repair genes; causing the resistance to natural and inherent death mechanisms embedded in cells (apoptosis) and dysregulation of cell proliferation. These tumor malignant cells undergo changes in their metabolic activity and start to synthesize divergent chemical compounds as a result of certain gene or antigene activation that remained unexpressed in normal cells (1). Also, tumor markers can be divided into those which are present in tissues as intracellular substances and those which are released into the circulation and appear in serum (1). Therefore, in different types of specimen markers can usually be detected or measured as circulating tumor cells in peripheral blood, urine, stool, in lymph nodes, in bone marrow and other body fluids and tissues by monoclonal antibodies (4). Following the development of monoclonal antibodies, many new tumor markers have been discovered during the past two decades but markers for every type of cancer still have to be found. Different tumor markers are found in different types of cancer and levels of the same tumor marker can be altered in more than one type of cancer (5). Some are specific for a single type of cancer, while others can be found in many types of cancer, also sometimes in non-cancerous diseases (5). And not every person with cancer may have higher level of a tumor marker.

Various tumor markers can be detected and measured by different types of assays and methods. Most commonly applied methodologies are immunohistochemistry, fluorescent *in situ* hybridization, reversed transcriptase and polymerase chain reaction, and immunoassays (1, 4). Although these modern techniques are very sensitive in measuring tumor markers, some difficulties can arise due to influence of different technical factors by the applied methodology (6). Two primary technical considerations are critical when measuring a tumor marker. The first is which type of assay should be used. The second is the reproducibility of the chosen assay, from both a technical and an analytical perspective (6). Thus, different test procedures may yield different assay results.

2.1. Clinical significance of tumor markers

It is known that more than 11 million people are diagnosed with cancer every year and it is estimated that there will be 16 million new cases every year by 2020 (7). Tumor markers play a key role in the management of cancer disease by being invaluable tools in cancer detection, diagnosis, patient prognosis and treatment selection (8). Therefore, tumor markers are an essential part of everyday clinical practice and it is of great importance to establish their clinical significance by determining their prognostic and predictive values. Prognostic biomarkers give information about clinical outcome independent of the treatment effect, whereas predictive biomarkers provide information on response to a specific therapeutic

intervention and are associated with tumor sensitivity or resistance to that therapy. Therefore, this review will focus on the clinical usefulness of various tumor markers already in clinical practice as well as certain potential markers, giving a brief description of their prognostic and predictive significance in different malignancies.

Main and potential clinical uses of tumor markers include determining the risk for developing the disease, disease screening and diagnosis, distinguishing between benign and malignant processes or between different malignant processes, predicting response or resistance to specific therapies, surveillance after primary surgery, and monitoring disease status during and after therapy (9). The majority of serum tumor markers showed to be particularly applicable in treatment monitoring and detection of recurrence (10). In most cases, cancer can only be diagnosed by a pathologist from a biopsy but possible presence of tumor markers in both malignant and benign tumors can enhance the effectiveness of a biopsy (3). So, they are generally not diagnostic but they can be used to support the diagnostic process and give useful prognostic information. In addition, some markers are associated with a more aggressive course and higher relapse rate and have value in staging and prognosis of the cancer (3). Changes in tumor marker levels are used to follow the course of a patients' disease, to measure the effect of treatment, and to check for recurrence of certain cancers. Each tumor marker has a variable profile of usefulness for screening, determining diagnosis and prognosis, assessing response to therapy, and monitoring for cancer recurrence. They can also help diagnose the source of widespread cancer in a patient when the origin of the cancer is unknown.

When evaluating tumor markers for use in clinical practice, there are certain requirements that are essential for their clinical acceptance (6). They include determining utility of marker, evaluating magnitude of their effects, analyzing their reliability, considering their technical, analytical, and trials design issues (6). A lot of effort has been put into studying clinical potential for various tumor markers and some of them proved to have significant clinical application (11). Although improvements have been made to identify tumor marker that are detectable in peripheral blood, bone marrow, or lymph nodes to improve early detection and treatment strategy for different neoplasms, reliable prognostic and predictive markers are still widely needed (12).

Currently, a lot of hope has been put into the development of new molecular approaches including genetics, genomics, and proteomics that have so far, provided a greater understanding of the cancer disease pathways, the protein targets and the pharmacologic consequences of drug administration. Scientists are evaluating patterns of gene expression (known as genomics) for their ability to predict patients' prognosis or response to therapy (5). Based on the expression of a signature set of genes tumors could be classified into clinically relevant categories (13). These numerous multigene expression profiles aim to outdo traditional predictive and prognostic factors. So far, there have been

many improvements in developing technologies capable of reading the gene expression profile such as Genomic Health's Oncotype DXTM and Agendia's MammaPrintTM whose aim is to discover the signature that would differentiate between breast cancers at high risk of recurrence and more indolent tumors (14). Although MammaPrintTM was approved by the Food and Drug Administration (FDA) and Oncotype DXTM was included in the American Society of Clinical Oncology (ASCO) clinical guidelines, these multi-gene assays are still not part of mainstream clinical practice. Another newer approach, complementary to genomics is called proteomics and it is widely used today in basic cancer research (5). This technology looks at the patterns of all the proteins in the blood instead of looking at individual protein levels. Because classic molecular approaches cannot give complete insight to cancer pathogenesis, proteomics helps us to identify proteins involved in essential molecular processes of cell division, progression and cell death (15). Therefore, proteomics enables the discovery of new drug targets and can reveal mechanisms of drug action, toxicity and resistance for the purpose of developing better cancer screening and treatment options (15). Also, it may help to identify new proteins that serve as tumor markers in early stages and to predict the effectiveness of treatment and probability of recurrence (5). These new testing methods are still in the early stages of development. Although proteomics plays an important part in clarifying cancer pathogenesis signaling pathways, none of the new drugs discovered by this technology are in routine use at this time.

So far, classical clinicopathological features such as tumor size, histological subtype and grade, lymph node metastases, and lymphovascular invasion have been used as a part of standard routine clinical practice indicating patient prognosis. These features have been integrated into TNM (tumor size, nodes, metastasis) system based on which tumor stages can be determined that have major prognostic value. Only a handful of tumor markers have been used in routine clinical practice but due to recent technical development new potentially valuable tumor markers in clinic have emerged that are still being investigated, such as numerous genetic markers, cytogenetic and cytokinetic markers, epigenetic biomarkers, circulating protein markers, hypoxic markers, cells as biomarkers, viral biomarkers (16, 17).

3. TUMOR MARKERS FOR BREAST CANCER

3.1. Cancer antigen (CA) 15-3

Cancer antigen (CA) 15-3 is a member of MUC-1 family of mucin glycoproteins that is well expressed on the apical surface of most polarized epithelial cells of different organs (10). CA 15-3 marker is a high molecular weight glycoprotein, localized in the cell membrane and detectable in serum. Generally, CA 15-3 is regarded as the most specific and sensitive serum tumor marker among MUC-1 family (18).

Although there is still conflicting data concerning the prognostic value of CA 15-3 marker but so far, it has

shown the strongest prognostic value among serum tumor markers for breast cancer (10). Preoperative elevated levels of CA 15-3 are an indicator of adverse effects in breast cancer patients, and CA 15-3 can be used as prognostic factor for both disease-free survival (DFS) and overall survival (OS) (19, 20). Serial serum determinations of CA 15-3 together with carcinoembryonic antigen (CEA) and serum human epidermal receptor protein 2 (HER 2) concentrations (with tissue overexpression) also proved to be useful tools in the prognostic evaluation of patients with primary breast cancer (20). Elevated CA 15-3 level can be a marker of enhanced risk of recurrence and mortality in both early- and advanced stage breast cancer (21). In some studies the prognostic impact of CA 15-3 was independent of tumor size and axillary nodal status and even CA 15-3 was found to be prognostic in lymph node-negative breast cancer patients (22, 23). Also, CA 15-3 has very important predictive value as well. The antigen was proven to be useful in the monitoring of response to either endocrine or cytotoxic routine therapy (21). Studies showed that elevated CA 15-3 levels predict a poor response to primary chemotherapy in locally advanced breast cancer (24). Also, post chemotherapy elevated CA 15-3 level together with the presence of lympho-vascular invasion and HER 2 positivity can predict a reduced DFS following treatment in locally advanced breast cancer (24).

CA 15-3 together with CEA currently represents the most used tumor markers for breast cancer in clinical practice but increased levels of CA 15-3 can also be found in cancers of ovary, lung, and prostate, as well as noncancerous conditions such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis (21).

3.2. Cancer antigen (CA) 27.29

CA 27.29 is a soluble form of glycoprotein MUC-1 located in the cell membrane, as well as CA 15-3, that is overexpressed in tumors involving glandular epithelial cells, such as breast tumors. It is another marker used to follow patients with breast cancer during or after treatment (10). It is newer but similar test to CA 15-3 for metastatic breast cancer detection and monitoring. Some studies point out that CA 27.29 has superior sensitivity and specificity, and that CA 27.29 would supplant CA 15-3 as the preferred tumor marker in breast cancer.

The CA 27.29 level is elevated primarily in metastatic breast cancer and in approximately one third of women with early-stage breast cancer (stage I or II) and in two thirds of women with advanced-stage disease (stage III or IV) (25). However, CA 27.29 has no role in screening or diagnosing the malignancy in the earliest stages of breast cancer. CA 27.29 is most frequently used to monitor the effectiveness of treatment in stage IV breast cancer, but its prognostic role is still not clear. Therefore, a multicenter German SUCCESS trial was conducted to evaluate the prognostic relevance of CA 27.29 marker in primary breast cancer patients before adjuvant chemotherapy (26). Serum CA 27.29 levels correlated with tumor size and nodal status indicating the independent prognostic significance in primary disease (26). Nevertheless, further follow-up of the

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SUCCESS-trial results are needed, especially the evaluation of CA27.29 blood level at follow-up examination after chemotherapy, which will hopefully clarify the prognostic relevance of this marker (27). Main clinical use of CA 27.29 marker lies in following response to therapy in patients with metastatic breast cancer and predicting recurrent breast cancer. Therefore, changes in the values of CA 27.29 marker can reflect disease progression and response to therapy. One study also showed that CA 27.29 levels correlate with combined [18F]-fluorodeoxyglucose positron emission tomography/computed tomography scans and circulating tumor cells in following the response to treatment and disease progression in patients with metastatic breast cancer (28).

Levels of CA 27.29 can also be increased in cancers of the colon, stomach, kidney, lung, ovary, pancreas, uterus and liver, as well as non-cancerous conditions such as first trimester pregnancy, endometriosis, ovarian cysts, non-cancerous breast, kidney, and liver disease.

3.3. E-cadherin

Cadherin 1 or epithelial cadherin (E-cadherin), encoded by CDH1 gene, is a member of cadherin family of Ca²⁺-dependent cell-cell adhesion glycoproteins. Inactivating CDH1 mutations lead to downregulation of E-cadherin protein, resulting in decreased cellular adhesion. This process can contribute to cancer progression by uncontrolled cell proliferation, invasion and metastasis (15). CDH1 mutations are found in breast, gastric, colorectal and ovarian cancer (8). Loss of E-cadherin function or expression has been associated with the development of metastasis and worse prognosis in patients with breast cancer. Aberrant expression is more frequent in invasive ductal carcinoma than in invasive lobular carcinoma, pointing to the different role of E-cadherin in various histological types of breast cancer (6).

3.4. Estrogen and progesterone receptors

Estrogen receptor (ER) belongs to the steroid nuclear receptor family and it is an estrogen-dependent transcriptional factor that regulates growth, development, differentiation and homeostasis by binding to the estrogen response element in DNA to modulate transcription of target genes. ER consists of two isoforms, ER- α and ER- β where ER- α is regarded as traditional estrogen receptor responsible for mammary gland development and tumorigenesis (29). But recent studies showed that ER- β has wider tissue distribution than ER- α and that it may be a prognostic marker, as well as a significant predictor of response to treatment in a variety of human cancers (30). Progesterone receptor (PR) is also an intercellular steroid nuclear receptor that mediates the action of progesterone via two isoforms termed A (PRA) and B (PRB) (31). The expression of the PR is strongly dependent on the presence of ER though in rare cases PR can be seen in the estrogen-negative tumors (32). ER and PR are found in the nucleus of breast and uterine tissues (10).

Overexpression of ER and PR is a significant prognostic marker in assessing the clinical outcome of

breast cancer patients. Generally, ER/PR-positive breast cancers have slower tumor growth, better differentiation, lower histology grade, DNA ploidy, and therefore are indicators of better overall prognosis (31, 33). On the other hand, ER/PR-negative tumors display more aggressive disease with amplification of HER2, c-Myc, and Int2 oncogenes, and mutations of the p53 tumor suppressor gene (34). However, certain limitations exist that make the use of ER as a prognostic factor somewhat controversial. Although ER positive patients have better outcome than ER negative patients for the first 4-5 years after diagnosis, the prognostic value of ER after that period becomes minor (35). Also, it has been shown that ER status has impaired prognostic significance in lymph node negative patients (35). Regardless of these limitations, ER can help in assessing the outcome in breast cancer patients when combined with classical clinical prognostic factors (36). Furthermore, it is considered that hormone status has a positive predictive role in breast cancer patients' response to hormonal therapy in the early and advanced stage of disease (37). Cancers with positive receptors are much more likely to respond to hormonal therapy such as tamoxifen or aromatase inhibitors, which bind to the receptors blocking the action of estrogen. Also, PR has shown to be more sensitive indicator than ER in predicting effective responsiveness to endocrine therapy in breast tumor patients (1). If hormone receptors are positive it is necessary to apply a specified kind of hormone therapy for patients with early, as well as advanced stage of breast cancer disease. Therefore, it is obligatory to determine hormone receptors for every breast cancer patient with methodology provided by ASCO recommendations and the College of American Pathologists (38). A low cut-off point >1% of positive ER and PR patients already represents a basis for applying hormone therapy, especially for metastatic disease (39) and in 2010 ASCO guidelines recommended a cut-off point >1% to define ER positivity (38).

Except in breast cancer, positive ER and PR status is also found in gynecological malignancies and a variety of other tumor types, including those of thyroid origin.

3.5. Human epidermal receptor protein 2 (HER 2)

HER 2 oncogene protein is a transmembrane glycoprotein from the epidermal growth factor receptor (EGFR) family with intracellular tyrosine kinase activity that is encoded by the HER 2 proto-oncogene (40). The HER 2 gene is either amplified or overexpressed in 15–30% of invasive breast cancers (10).

HER 2 gene amplification or protein overexpression proved to be of significant clinical utility by being a valuable prognostic and predictive marker in breast cancer patients. HER 2 overexpression and/or gene amplification represents an independent prognostic marker of clinical outcome indicating worse prognosis in both node-negative and node-positive breast cancer patients (35). Although HER 2 gene amplification or overexpression correlates with an adverse outcome in patients with breast cancer, HER 2 should not be used

alone for determining outcome in patients with breast cancer (41). HER 2 is strong predictor for non-responsiveness to hormonal and other chemotherapy treatments. As a predictive marker, HER 2 overexpression showed to be relatively resistant to certain chemotherapy regimens (42). Especially, HER2 status has been shown to predict sensitivity to anthracycline-based chemotherapy regimens (43).

But most importantly, HER 2 should be assayed in order to select patients for treatment with trastuzumab (Herceptin®), a humanized monoclonal antibody that works against the HER 2 receptor on breast cancer cells thereby improving response rate and survival (44). Therapy with Herceptin is widely applied in patients with tumor positive HER 2 at an early stage, as well as advanced stage of breast cancer. Therefore, determination of HER 2 status is obligatory for every breast cancer patient with methodology provided by ASCO recommendations (45). However, some HER 2 positive tumors have a primary or acquired resistance to trastuzumab.

3.6. uPA and PAI-1

The urokinase plasminogen activator (uPA) system consists of the serine protease uPA; its glycolipid-anchored receptor uPAR, located on the cell membrane; and its 2 serpin (serine protease) inhibitors: plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2) (46). uPA is a trypsin-like protease that converts the zymogen plasminogen into active plasmin and whose primary physiological inhibitor is PAI-1, a single-chain glycoprotein often associated with the extracellular matrix.

Studies showed that uPA and PAI-1 play a critical role in cancer growth, invasion and metastasis (47). Their overexpression has been consistently related to tumor aggressiveness and adverse clinical outcome in breast cancer patients (48). Patients with high uPAI and PAI-1 levels have worse DFS and OS than patients with low levels of these markers (49). Both a randomized prospective trial and a pooled analysis have shown that uPA and PAI-1 are potent and independent prognostic factors in breast cancer (50, 51). Furthermore, uPA and PAI-1 biomarkers have reached the highest level of evidence (level-of-evidence-1) regarding their clinical utility in breast cancer (52). This prognostic impact of uPA and PAI-1 has been shown in both lymph node negative and lymph node-positive breast cancer patients (48). uPA and PAI-1 biomarkers have been used to determine disease risk and to select node-negative breast cancer patients who do not need, or are unlikely to benefit from, adjuvant chemotherapy, i.e., patients with low levels of uPA and/or PAI-1 (50). Also, uPA and PAI-1 have been reported to predict response or resistance to specific therapies in patients with breast cancer. High levels of these proteins are associated with resistance to hormone therapy in advanced breast cancer, but correlate with enhanced benefit from adjuvant CMF therapy in early breast cancer (53). uPA/PAI-1 combination showed to be predictive for response to anthracycline-based chemotherapy and first line endocrine therapy (54).

uPA and PAI-1 also serve as biomarkers in other malignancies besides breast cancer, such as gastrointestinal cancers, gynecological cancers, bladder, gliomas, sarcomas (48).

3.7. Ki-67

Well-established marker for determining cell proliferative activity and biological aggressiveness is a nuclear protein Ki-67 (55). Antigen Ki-67 is present in all proliferating cells and expressed in all active phases of the cell cycle except G0 (56). Ki-67 is a nuclear nonhistone protein with two isoforms, “large” (359 kD) and “small” (320 kD) Ki-67 protein, that are found mainly in the nucleolar cortex and in the dense fibrillar components of the nucleolus (57).

The use of Ki-67 as a predictive and prognostic marker in breast cancer has been widely investigated. High level of Ki-67 marker is a sign of poor prognosis and it is considered to be a significant independent prognostic factor for breast cancer (58). Ki-67 gene is also included in the Oncotype DX™ assay used to predict the risk of recurrence and the extent of chemotherapy benefits in women with node-negative, ER-positive breast cancer (59, 60). Many studies showed that high Ki-67 labeling index (the percentage of cells with expressed Ki-67) correlates well with other prognostic breast cancer factors such as negative ER and PR status (61-63), positive HER-2 (62, 64), and p53 overexpression (62, 63). Also, Ki-67 expression showed to be a prognostic factor for both OS and DFS (65). Nevertheless, the existing ASCO guidelines do not include Ki67 in the list of required routine biological markers due to lack of standard methodology and accepted cut-off points for proper assessment of this marker. Regarding Ki-67 as a predictive factor, several small studies have reported that a high Ki-67 labeling index predicts better response to neoadjuvant (primary) chemotherapy in breast cancer patients (66, 67). However, the predictive value of a high Ki-67 labeling index for response to adjuvant chemotherapy is still unclear.

By using gene microarray analysis, breast cancer, as a heterogeneous disease, can be classified into four different subtypes: basal-like subtype, luminal A, luminal B, and HER 2 positive. Besides gene analysis, Ki-67 index is also used to distinguish breast cancer subtypes along with the expression of ER, PR and HER 2 (68). Since these subtypes have different prognostic and possible therapeutic implications, it is of great importance to make the classification in order to determine and apply the adequate therapy. Luminal B tumors have higher proliferation than luminal A tumors therefore, Ki67 labeling index may serve as a clinically valuable biomarker for the luminal B subtype. Although the constant cut-off point and scoring protocol have not yet been standardized when considering the prognosis for breast cancer patients of all subtypes, Cheang and colleagues (68) suggested that the most appropriate Ki-67 index cut-off point to distinguish luminal B from luminal A tumors was 13.25%. Breast tumors with Ki-67 level less than 13.25% were classified as luminal A and tumors with levels above 13.25% were classified as luminal B subtype with worse prognosis for both breast

cancer recurrence and death (68). Keam and colleagues suggested two subgroups of triple negative breast cancer based on the Ki-67 expression that might exist, each with differential response and prognosis following neoadjuvant chemotherapy (69).

High levels of Ki-67 have been also associated with several other malignancies (bladder cancer, prostate cancer, hepatocellular carcinoma, thyroid carcinoma) (70-73).

3.8. BRCA1 and BRCA2

BRCA1 and BRCA2, as genetic tumor markers, belong to a class of genes known as tumor suppressors that regulate transcription, inhibit cellular proliferation and repair DNA, thereby maintaining genomic integrity and preventing dangerous genetic changes in normal cells (74). The BRCA1 gene is located on the long arm of chromosome 17 (17q21) and BRCA2 gene is located on chromosome 13q12 and mutations on these genes are involved in breast and ovarian carcinogenesis (75). These mutations can be inherited therefore, these markers, as cancer predisposing genes, are used to assess the risk in individuals with a familial history of breast cancer. Studies showed that women who carry a germline mutation in BRCA1 have a cumulative lifetime risk of 50%–85% of developing breast cancer and 12%–60% of developing ovarian cancer (76).

Breast cancers with BRCA1 or BRCA 2 mutations often have a high nuclear grade, poorly differentiated morphology, negativity to ER/PR/HER2, positivity to cytokeratins, overexpression of cyclin E, low expression of p27KIP1, and p53 mutations (77). BRCA1/2 mutations have been associated with a poor prognosis in breast cancer patients within an Ashkenazi Jewish population (78). Recent studies showed that breast cancer patients who have BRCA1/2 mutations had lower survival rate, were less likely to express ER/PR/HER2, and had increased recurrence compared with patients without these mutations, especially those with BRCA1 mutations (79, 80). Prognostic studies indicated that BRCA1 mutations have a similar or worse outcome than BRCA2 mutations (81). Although non-BRCA1/2 hereditary breast cancers express a less aggressive profile than BRCA1/2 related cancers (82), the prognostic studies of these genetic markers often express conflicting results. In some cases, BRCA1/2 mutations have failed to demonstrate the prognostic values in breast cancer patients (34). Preclinical breast cancer studies showed that BRCA1 expression can modulate cellular response to chemotherapy by predicting response to DNA-damaging agents and taxane-based chemotherapy. Decreased BRCA1 expression enhances cisplatin sensitivity but leads to resistance to paclitaxel and vinorelbine while the opposite phenomenon is observed in the presence of normal or high levels of BRCA1 (83). Furthermore, several retrospective breast cancer studies have confirmed that carriers of BRCA1 mutations gained more benefit from DNA damage-based chemotherapy (84). Nevertheless, in breast cancer, little is known regarding clinically important differences in response to chemotherapy between BRCA1/2 mutation carriers and

non-carriers, and between different chemotherapeutic regimens within existing series of BRCA1/2 mutation carriers.

Mutations in the BRCA1 gene are widely prevalent in patients with familial breast and ovarian cancer while BRCA2 is associated with familial cancers of the female and male breast and, to a lesser extent, the ovaries. Mutations of BRCA2 is involved in a male breast cancer and other cancers of prostate, pancreas, bladder, non-Hodgkin's lymphoma, basal cell carcinoma, and fallopian tube tumors (34).

3.9. Oncotype DXTM and MammaPrintTM

So far, based on these clinicopathological factors, clinicians have not been able to determine which patients will benefit from adjuvant therapy. Gene expression profiling has shown promise to distinguish between patients at low and high risk for developing distant metastases and identify those who are likely to benefit from adjuvant therapy (85). Since 2006 European Commission launched a new prognostic RNA-based tool that has the potential to greatly improve risk assessment and treatment decision making for early breast cancer. The MINDACT trial (Microarray In Node negative Disease may Avoid ChemoTherapy) is a prospective, 6000-patient, phase III randomized, multicentric trial that tries to validate the superior performance of the Amsterdam 70-gene expression profiler MammaPrintTM, discovered at the Netherlands Cancer Institute (86). Together with the second most important prognostic tool for breast cancer; the TAILORx (Trial Assigning Individualized Options for Treatment [Rx]) trial, (sponsored by Oncotype Dx, Genomic Health, USA) (59), these studies are trying to determine the clinical application of genomics in order to implement its use in clinical practice later on.

Development of the Oncotype DXTM molecular profiling assay was inspired by the desire to determine the prognosis with lymph node negative, ER–positive tumors receiving tamoxifen (59). It is a genomic noninvasive assay that analyses a panel of 21 genes within the tumor associated with proliferation and endocrine response in order to establish how a cancer is likely to grow and respond to treatment. The levels of expression of 16 outcome-related genes and 5 reference genes are measured by multiplex reverse transcription-PCR based on which a recurrence score is calculated that represents a number between 0 and 100 that corresponds to a specific likelihood of breast cancer recurrence within 10 years of the initial diagnosis (59). The lower the score is, it is the less likely for cancer to recur and vice-versa. Oncotype DxTM has been included in the 2007 ASCO clinical guidelines (87). Oncotype DXTM is both a prognostic test, since it provides more information about how likely (or unlikely) the breast cancer is to come back, and a predictive test, since it predicts the likelihood of benefit from chemotherapy treatment. The recurrence score has been found to predict distant recurrence independent of age and tumor size and is predictive of OS and the magnitude of chemotherapy benefit in node-negative, ER-positive breast cancer (60).

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Mamma Print™ is another molecular profiling approach that is currently being tested in the MINDACT clinical trial whose main objective is to better select patients for adjuvant chemotherapy in node-negative breast cancer. The trial also aims to address the questions related to adjuvant treatment of breast cancer by comparing anthracycline-based chemotherapy regimens to a docetaxel-capecitabine regimen, and investigate the efficacy and safety of 7 years single agent Letrozole to the sequential strategy of 2 years of Tamoxifen followed by 5 years of Letrozole. So far, MINDACT trial showed encouraging results. The 70-gene signature was compared with St Gallen and National Institutes of Health criteria and it seems to more accurately determine the risk of relapse for individual breast cancer patients than the traditional clinical-pathological criteria currently used (88, 89). This profiler showed to be efficient in identifying high-risk patients who would have needed chemotherapy, but had a higher accuracy in identifying low-risk patients who could have been spared adjuvant chemotherapy (90). High-risk patients showed a higher risk of developing distant metastases than did the high-risk patients classified by the St Gallen or National Institutes of Health criteria (91). The 70-gene signature also showed significant prognostic usefulness for distant DFS and OS (90). Nevertheless, during this type of research many issues have emerged concerning ethical and legal facts such as mandatory collection of biological material within clinical trials, issues of tissue ownership, consenting and re-consenting patients, and intellectual property rights (92).

4. TUMOR MARKERS FOR GASTROINTESTINAL CANCER

4.1. Cancer antigen (CA) 19-9

Cancer antigen (CA) 19-9 is a sialylated Lewis blood-group antigen of the MUC 1 protein found mostly on the luminal cell membrane surface and in luminal content of the glandular structure of different organs including pancreas, biliary tract, as well as gastric, colonic and salivary epithelia (93).

Although the CA 19-9 test was first developed to detect colorectal cancer, it is more sensitive (85%) to pancreatic cancer. CA 19-9 serum antigen is a widely used diagnostic and prognostic biochemical marker in pancreatic cancer patients. It has proven to be clinically useful in diagnosis, assessment of resectability, and monitoring of pancreatic cancer progression and prognosis (94-96). Numerous studies have shown that increases in CA 19-9 are associated with poor outcome and survival, therefore serum CA 19-9 concentration showed to be an independent prognostic marker (97). Some studies have revealed that pretreatment level of CA 19-9 can be used as a prognostic indicator in patients with advanced pancreatic cancer (98, 99). Other studies showed that postoperative CA19-9 is a better prognostic marker than preoperative CA 19-9 (100). Moreover, postoperative CA 19-9 value >37 U/ml was shown to be an independent significant prognostic factor for identifying patients with poor prognosis (100). CA 19-9 showed to be of predictive value for prognosis, response, and detecting recurrence of pancreatic cancer in patients

undergoing combined radiochemotherapy (101). A multivariate prognostic analysis indicated that preoperative CA 19-9 level is an independent prognostic factor in advanced stage colorectal cancer (102). Another study demonstrated a significant predictive value of CA 19-9 in response to chemotherapy and bevacizumab (103).

Due to its high specificity, CA 19-9 plays an important role in the diagnosis, therapeutic monitoring and monitoring of the course of gastrointestinal carcinomas, however increased levels of CA 19-9 can also be found in patients with nonmalignant inflammatory diseases, such as cholecystitis and obstructive icterus, cholelithiasis, cholecystolithiasis, acute cholangitis, toxic hepatitis and other liver diseases (104).

4.2. Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen is a glycosyl phosphatidyl inositol-cell surface anchored oncofetal cytoplasmic glycoprotein that is expressed in normal mucosal cells and overexpressed by adenocarcinomas. CEA, as an intracellular adhesion molecule, is the preferred tumor marker for patients with colorectal (105) and breast cancer where it is considered an independent prognostic tool together with CA 15.3 and HER 2 (21). Over 50% of persons with breast, colon, lung, gastric, ovarian, pancreatic, and uterine cancer have elevated levels of CEA.

Elevated serum levels of CEA indicate a poor outcome in colorectal cancer (CRC) and the high risk of cancer recurrence. Various studies demonstrated that elevated preoperative CEA level is an independent risk factor and prognostic marker for poor survival in CRC (106). Preoperative serum CEA showed to be a reliable predictive factor of recurrence after curative surgery and a useful indicator of the optimal treatment after CRC resection (107). An elevated postoperative CEA level is also an adverse prognostic indicator after resection of colorectal liver metastases (108). The higher the CEA level at the time CRC is detected, the more likely it is that the cancer is advanced. A study demonstrated that CEA kinetic can be used to predict response to chemotherapy in patients with metastatic CRC (109). Nonetheless, CEA lacks sensitivity and specificity and thus in some cases can be a poor prognostic and predictive factor.

An increased value of CEA has been observed in cancer of colon, rectum, lung, breast, liver, pancreas, prostate, stomach, and ovary but also in benign liver, gastric, intestinal and breast diseases, pulmonary infection, emphysema, and renal failure (3).

4.3. Alpha-fetoprotein (AFP)

Cytoplasmic protein AFP is the major glycoprotein of fetal serum but its blood levels fall to an undetectable level after birth. AFP is considered as a golden standard amongst diagnostic markers for hepatocellular carcinoma (HCC) with its levels abnormal in 80% of patients (110). Diagnose of HCC can be confirmed if AFP concentration is >400-500 micro g/L because it is accepted as the optimal decision point to discriminate HCC from chronic liver disease (110).

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Although AFP is not sensitive or specific enough for early detection of the HCC, it is suggestive and useful as a prognostic marker where elevated AFP concentrations are indicators of unfavorable prognosis and worse clinical outcome (111). Also, high AFP value has been correlated with major clinicopathologic factors (112). In recent decades, research has also been focusing on AFP related parameters, AFP mRNA and AFP glycoforms that expressed prognostic value with its potential use in diagnosis and monitoring of HCC patients (113). AFP elevation has been associated with HCC progression and with increased risk for early recurrence and poor prognosis after hepatectomy (114). Various studies showed that AFP response is an independent prognostic factor for survival in HCC patients undergoing systemic chemotherapy (115, 116). AFP proved to be indicative of tumor activity and it can be used to assess response to therapy (115). Another study also demonstrated that AFP response is a reliable predictor of tumor response, time-to-progression, progression-free survival, and OS in HCC patients treated with locoregional therapies (117). Furthermore, a recent study indicated that an early AFP response is a useful surrogate marker to predict treatment response and prognosis in patients with advanced HCC who receive antiangiogenic therapy (118).

The primary malignancies associated with AFP elevations are HCC and certain rare types of ovarian and testicular cancer called yolk sac tumor or mixed germ cell cancer (110). Slightly elevated AFP levels are also found in several other carcinoma types (e.g., gastric carcinoma, testicular carcinoma, lung cancer and pancreatic cancer) and non-tumoral conditions (e.g., chronic hepatitis and liver cirrhosis). Pregnancy is also associated with elevated AFP levels, particularly if the pregnancy is complicated by a spinal cord defect or other abnormality.

4.4. K-Ras

Ras proteins are a family of GTPases which are involved in signaling pathways controlling cell proliferation and differentiation. K-Ras is one of the three cellular Ras genes with the most frequent mutations which make it an ideal target for cancer treatment (119). The human K-Ras gene encodes a small G-protein that functions downstream of EGFR induced cell signaling. Mutations on K-Ras gene activate the signaling pathways involved in the development of CRC. Alternations mostly occur in codons 12 and 13 in exon 2 of the K-Ras gene (~82% and ~17% of all reported K-Ras mutations) playing a major role in the progression of CRC (120), while mutations in codons 12, 13, and 61 are potential biomarkers in lung cancer (121).

K-Ras mutations have been independently associated with worse outcome especially in stage II and III of the CRC disease (122). Studies demonstrated an association of K-Ras mutation with worse outcomes and accelerated progression of colorectal liver metastasis in patients having undergone surgical resection (123). K-Ras mutations on codon 12 are indicators of unfavorable outcomes, especially in those individuals with advanced disease. Importantly, K-Ras mutations have been associated

with clinical resistance to EGFR-specific antibody therapy. Many studies showed that K-Ras mutation is a significant predictor of a very poor response to panitumumab (Vectibix) and cetuximab (Erbix) therapy in CRC patients (124). K-Ras mutations have almost ideal predictive marker characteristics when evaluating EGFR-specific agents because the mutations are limited to a small part of the gene and are easily detected, and the negative predictive value is high (125).

Although K-Ras mutations have been found in pancreatic, endometrial, biliary tract, lung, and cervical cancers (126), their clinical utility has been mainly studied in CRC.

4.5. Microsatellite instability (MSI)

MSI is a type of genetic instability characterized by length alterations within simple repeated microsatellite sequences and occurs in tumors with deficient mismatch repair due to inactivation of one of the four mismatch repair genes: MSH2, MLH1, MSH6 and PMS2 (127). MSI occurs in the majority of Lynch syndrome (hereditary non-polyposis colorectal cancers) associated cancers and in a subset of sporadic cancers such as colorectal, gastric and endometrial cancers (128).

MSI status has been studied as both a prognostic and predictive marker in colorectal cancer and so far, studies have revealed its potential critical significance for both patient prognosis and prediction. MSI is positive in approximately 20% of patients with CRC and it is associated with favorable outcome, less tumor recurrence and significant survival advantage (129). High frequency of MSI has shown to be an independent prognostic variable in colon carcinoma patients correlating with resistancy to chemotherapy (130). MSI has shown to be a predictive marker of response to the treatment with 5-fluorouracil (5-FU)-based chemotherapy (131). Eastern Cooperative Oncology Group 5202 trial is further exploring this association whose aim is to select stage II colorectal cancer patients for adjuvant therapy based on MSI and 18q loss-of-heterozygosity status.

4.6. Adenomatous polyposis coli (APC) and beta-catenin

The APC is a tumor suppressor gene that regulates the cell division cycle by keeping cells from growing and dividing too fast or in an uncontrolled way. Its protein product plays a critical role in cell migration and adhesion, transcriptional activation, and apoptosis. Familial adenomatous polyposis (FAP), caused by the mutation in APC gene, is an inherited condition in which benign polyps in the epithelium of the large intestine can transform into colon cancer. In carriers of APC inactivating mutations, the risk of colorectal cancer by age 40 is almost 100% (125). More than 700 APC mutations have been identified in patients with FAP. The most common mutation is the deletion of five nucleotides that changes the sequence of amino acids at protein position 1309. Mutations in APC lead to loss of beta-catenin regulation, altered cell migration and chromosome instability (125).

Beta-catenin, a central molecule of the Wnt-signaling pathway, is a part of protein complex that

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constitute adherens junctions and regulate cell growth and adhesion between cells by transmitting the contact inhibition signal. The CTNNB1 gene that codes for beta-catenin protein can function as an oncogene, which mutations correlate with increased tumor cells proliferation. CTNNB1 mutations are found in colorectal cancer, medulloblastoma and ovarian cancer. Mutations in beta-catenin and APC, inherited or sporadic, can have a critical role in early development of colon cancer (125).

5. TUMOR MARKERS FOR GYNECOLOGICAL CANCERS

5.1. Cancer antigen (CA) 125

Cancer antigen (CA) 125 is a high molecular weight mucin glycoprotein located in the cell membrane of fetal amniotic and coelomic epithelium and in adult tissues derives from the coelomic (mesothelial cells of the pleura, pericardium, and peritoneum) and Mullerian (tubal, endometrial, and endocervical) epithelia (132). CA 125 is raised in approximately 90% of patients with advanced epithelial ovarian cancer and in 50% of early ovarian cancers patients with values above 35 U/mL (133). Measurement of the serum level of the CA 125 antigen has become a standard component of routine management of women with advanced ovarian cancer (134).

Tumor marker CA 125 has been widely accepted as a predictive and prognostic factor in CA 125 positive ovarian cancers. Although different studies sometimes showed contradictory results, serum CA 125 level is regarded a strong prognostic factor for OS and progression free survival in ovarian cancer. Elevated postoperative CA 125 serum concentrations showed to be an independent prognostic factor in patients with invasive ovarian cancer indicating tumor recurrence, worse clinical outcome and poor survival (135). Also, during the course of chemotherapy CA 125 level can serve as an indicator of clinical outcome (136). A decrease in CA 125 level is related to a positive response to cancer therapy. CA 125 tumor marker half-life and doubling time can be used for the evaluation of clinical response and follow-up of patients with ovarian cancer (137). Serum CA 125 half-life during early chemotherapy showed to be an independent prognostic factor for both the response and the survival of patients with advanced epithelial ovarian cancer (138).

CA 125 can be elevated in many benign conditions including pregnancy, leiomyomata, ovarian cysts, endometriosis, appendicitis, and diverticulitis (133). CA 125 can also be elevated in other cancers such as uterine, colon, lung, or pancreas (133).

5.2. Human chorionic gonadotropin (HCG)

HCG belongs to the glycoprotein hormone family that also comprises luteinising hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. HCG consists of alpha and beta subunits and is produced by the syncytiotrophoblastic cells of the placenta and is elevated in pregnancy. HCG blood levels are elevated in patients with some types of testicular and ovarian cancers (germ cell tumors) and in gestational trophoblastic disease,

mainly choriocarcinoma (35, 115, 139, 140). In patients with extragonadal disease or metastasis at the time of diagnosis, highly elevated HCG values can be used in place of biopsy to establish a diagnosis of nonseminomatous germ cell tumor.

Increased serum HCG levels and its metabolites are generally regarded as a sign of a poor prognosis and it has been suggested that HCG beta might directly modify the growth of the cancer, leading to a worse outcome. HCG levels, in a patient with a testicular cancer, above 50,000 U/mL at initial diagnosis portend a poor prognosis, with a five-year survival rate of 50% (139). HCG in serum and urine provides a strong independent prognostic factor in ovarian carcinoma, and its prognostic value is similar to that of grade and stage (140, 141). Kinetics of early serum tumor marker HCG decline during chemotherapy was shown to predict survival in patients with poor prognosis of non-seminomatous germ cell tumors; therefore it is an important predictor of PFS and OS (142). Also, this kinetic population approach, in another study, has indicated that HCG clearance is the major independent predictive marker for chemotherapy resistance risk in low-risk gestational trophoblastic neoplasias (143). Also, kinetic modeling has shown to be a promising method for analyzing HCG together with AFP in nonseminomatous germ cell tumor patients treated with chemotherapy (144). Research showed that by calculating individual areas under the curve (AUC) for HCG and AFP we can predict more accurately the disease progression risk (144).

5.3. Squamous cell carcinoma antigen (SCC-Ag)

SCC-Ag is a sub-fraction of the tumor antigen TA-4 glycoprotein that comprises two nearly identical proteins, SCC-1 and SCC-2 and it belongs to the family of serine protease inhibitors (145). SCC-Ag is a serpin associated with squamous cell carcinomas of different organs, but most commonly used as tumor marker for cervical cancer. SCC-1 and SCC-2 reside in the cytosol of squamous cells, but are present in the serum of patients with advanced squamous cell carcinomas, mainly due to a passive release rather than an active secretory process into the circulation (146).

Serum SCC-Ag levels were found to be elevated in 28–88% of patients with squamous cell cervical cancer and they correlate with tumor stage, tumor size, depth of stromal invasion, lymph-vascular space status, parametrial involvement and lymph node status (147). High preoperative serum SCC-Ag levels were associated with poor prognosis and different studies indicated that pre-treatment SCC-Ag is an independent risk factor of poor DFS and OS (148–150). Nevertheless, several other studies reported that it has no prognostic value, therefore the clinical prognostic significance of pre-treatment serum SCC level is still considered controversial. Furthermore, analysis of decline in serum SCC-Ag levels during treatment seems to be indicative of tumor response to treatment and as well as outcome of patients (147). Studies showed that 70–86% of cervical cancer patients with recurrent disease had elevated SCC-Ag levels at some time during follow-up (151). Also, SCC-Ag showed to be a

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relatively good method for the detection of disease recurrence in patients with cervical cancer who were treated by concurrent chemoradiotherapy (152). According to some authors serum SCC assay during the follow-up does not improve the cure rate of patients who will ultimately develop a recurrence (147).

Elevated levels have been found in patients with squamous cell carcinomas of the vulva, vagina, head and neck, esophagus, anal canal and lung, as well as in patients with certain benign diseases (153-155).

6. TUMOR MARKER FOR PROSTATE CANCER

6.1. Prostate-specific antigen (PSA)

PSA is a chymotrypsin-like serine protease (kallikrein family) present in the cytoplasm of prostate gland cells. This small glycoprotein is secreted by the epithelial cells of the prostate gland, as well as the epithelial lining of the periurethral glands, and it is responsible for the liquefaction of seminal fluids. PSA is a prostate-specific tumor marker and can be measured in the serum as an "organ-specific marker" in 2 major isoforms: isoform 1 is complexed to alpha-1-antichymotrypsin and isoform 2 is uncomplexed free PSA (156, 157). PSA marker can also show elevated serum levels in a variety of other conditions such as prostatitis, benign prostate hyperplasia, and non-cancerous neoplasia.

PSA has been recognized as a screening tool to detect the presence of prostate cancer and to evaluate the treatment response (158). So far, PSA has been the only serum biomarker recommended by the American Cancer Society for use in the screening of malignancies (157). Despite the difficulties in establishing an optimal upper limit of normal PSA value 4 ng/mL had been generally accepted as a cut-off point for identifying prostate cancer risk. To increase PSA sensitivity and specificity in diagnosis of prostate cancer, various PSA parameters have also been used as prognostic indicators, such as PSA density, PSA velocity, PSA half-life, PSA nadir, PSA doubling time, PSA relating to age, and total-to-free PSA ratio. Their pretreatment values have been developed to predict pathologic stage, PSA recurrence, and DFS (159). Preoperative PSA velocity showed to be an independent factor for the prediction of relapse after radical prostatectomy (160). Both the preoperative PSA velocity and PSA doubling time were shown to be significant predictors of biochemical progression, clinical progression, and death from prostate cancer (161). PSA remains an important prognostic marker for prostate cancer patients indicating adverse outcome even among men with preoperative PSA level < 10 ng/mL (162). Also, recent clinical data support a new role for PSA in the determination of the risk of future prostate cancer (163). Furthermore, the predictive value of PSA kinetic to PSA doubling time was shown to be predictive to response and the duration of the response to deferred antiandrogen therapy in prostate cancer patients (164).

7. TUMOR MARKERS FOR LUNG CANCER

7.1. CYFRA 21-1

CYFRA 21-1 is a fragment of cytokeratin-19, an epithelial protein soluble in water. The highest CYFRA 21-

1 concentrations are found in lung cancer, mainly in non-small cell lung cancer (NSCLC) but it is also increased in several other malignancies including most gynecological or gastrointestinal tumors, mesotheliomas and urological malignancy (154, 165).

Compared with other tumor markers, CYFRA 21-1 showed to be the most sensitive tumor marker in lung cancer, with the highest concentrations in squamous tumors. CYFRA 21-1 can serve as a predictive and prognostic marker in lung cancer treatment. Studies have shown that high serum concentrations of CYFRA 21-1 are mainly related to tumor burden, and indicate a poor clinical outcome (166). A high preoperative CYFRA 21-1 level was a significant independent prognostic factor in patients with stage I NSCLC (167). Therefore, CYFRA 21-1 can be regarded as a strong prognostic marker in analyzing NSCLC outcome. Its clinical usefulness also includes aid in early diagnosis of recurrence, post-operative surveillance, and therapy monitoring in advanced disease, mainly in NSCLC (154, 165). CYFRA 21-1 also appears to be a reliable marker in predicting the response to chemotherapy for NSCLC. Studies demonstrated the great potential of CYFRA 21-1 for predicting the therapy response, especially during first line-chemotherapy in advanced NSCLC (168). CYFRA 21-1 also showed to be valuable for the individual management of patients with recurrent NSCLC, indicating tumor response after one cycle of chemotherapy (169). In addition, kinetics of CYFRA 21-1 was reported to be an independent prognostic marker for OS which may reflect the response to therapy in the long-term run (209).

7.2. Neuron-specific enolase (NSE)

NSE is an isoenzyme of the glycolytic pathway that is found only in brain and neuroendocrine tissue. NSE, the γ -subunit of the glycolytic enolase enzyme is localized in cytosol of neuroendocrine cells and overexpressed in neuroendocrine tumors (NETs). Among NETs it has been widely recognized as a biomarker for small cell lung carcinoma (SCLC) where 70-100% of SCLC demonstrated NSE overexpression (170). Elevated serum levels of NSE are noted in only 23-60% patients with NSCLC (171).

NSE marker can be measured by its serum concentration and tissue expression. Studies confirmed that NSE serum concentrations are important in differentiation between NSCLC and SCLC, and that it represents a more significant prognostic marker for SCLC patients (171). In general, elevated NSE level is considered to be an unfavorable prognostic factor, a negative prognostic index for survival both in SCLC and NSCLC patients (172). NSE concentrations also showed to correlate with the patients' extent of disease. While NSE serum level is an indicator of worse prognosis, positive NSE tissue expression in SCLCs seems to indicate longer survival of the patient (173). Studies on NSE in serum and tissue in pulmonary tumors indicate that the protein is important more as a prognostic than a diagnostic marker of neuroendocrine lung carcinomas (172, 173).

NSE can also be used to assess the potential for an individual patient to respond to various therapies. Lack

of normalization of NSE concentration during treatment is linked to poor prognosis irrespectively of clinical response (171). Serum NSE levels correlate very well with response to platinum-based chemotherapy that is the standard of treatment for patients with SCLC (170). Study showed that pretreatment NSE levels inversely correlated with time to progression and survival in SCLC patients. During treatment and pretreatment serum NSE levels showed to be independent prognostic predictors of time to progression and survival (174).

7.3. EML4-ALK fusion gene

In 2007 a novel recurrent gene fusion was described in NSCLC cells between the N-terminal portion of the protein encoded by the echinoderm microtubule-associated protein-like 4 (EML4) gene and the intracellular signaling portion of the receptor tyrosine kinase encoded by the anaplastic lymphoma kinase (ALK) gene (175). Small inversions within the short arm of chromosome 2p (involving 2p21 and 2p23) are responsible for the formation of these oncogenic fusion genes that occur approximately in 2 to 7% of all NSCLC patients (176). As an aberrant fusion gene in NSCLC, EML4-ALK encodes a cytoplasmic chimeric protein with constitutive kinase activity and several distinct EML4-ALK chimeric variants have been identified in lung cancer (177). A significant relationship was found between smoking and EML4-ALK positivity, and EML4-ALK fusions have also been associated with a lack of EGFR or K-Ras mutations, younger age, and adenocarcinomas with acinar histology (178-180).

EML4-ALK may serve as prognostic marker, as well as predictive marker for patients with metastatic NSCLC (181). Data showed that patients with EML4-ALK-positive NSCLC have a superior outcome compared with wild type (181). Furthermore, these ALK mutations are clinically susceptible to pharmacologic ALK kinase inhibition, therefore can serve as promising candidates for a therapeutic target as well as for a diagnostic molecular markers in NSCLC. EGFR tyrosine kinase inhibitors such as erlotinib (Tarceva) and gefitinib (Iressa) are used in EGFR-mutant NSCLC patients where they block activation of the signaling pathways for cell growth and survival. Recently, ALK positive tumors emerged as a second genetically defined subgroup of oncogene-driven lung cancer that is highly susceptible to targeted therapy. A recent study reported tumor shrinkage and disease stability when an oral ALK tyrosine kinase inhibitor crizotinib was used in patients who had NSCLC with these ALK gene rearrangements (176). Shaw and colleagues showed that response rate, time to progression and OS of patients with ALK genomic alterations who were treated with platinum-based chemotherapy, were similar to NSCLC patients harbouring EGFR mutations or those that were wild-type for both EML4-ALK and EGFR (182).

Activating mutations or translocations of the ALK gene have been identified in several types of cancer, including anaplastic large-cell lymphoma, neuroblastoma, inflammatory myofibroblastic tumor, and NSCLC (175, 183, 184).

8. CONCLUSION

Recently, the identification and usage of new tumor markers in clinical oncology have increased due to the expansion of detection techniques and our understanding of the disease processes. Some of tumor markers have shown to be of considerable clinical reliability and have been accepted into standard clinical practice. Currently available tumor markers can provide additional information regarding diagnosis and disease monitoring, estimation of patient prognosis and prediction of therapy response. Discovery and clinical application of new sensitive and specific markers, with diagnostic and prognostic values, could have a significant role in individual patient prognosis and treatment adjustment. Upcoming genomic and proteomic technologies are leading clinical oncology into a new era in which cancer diagnosis and treatment will be guided by the molecular attributes of every individual tumor and patient. Understanding of signaling pathways and genetic alterations in individual cancer enables identifying specific targets for new drugs and therapeutic strategies. These new molecular approaches showed to be quite promising in enhancing the efficacy of cancer management by providing tools for prediction of therapeutic response and facilitating the individualization and personalization of anticancer therapy.

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Abbreviations: FDA: Food and Drug Administration; ASCO: American Society of Clinical Oncology; TNM: tumor size, nodes, metastasis; CA: cancer antigen; DFS: disease-free survival; OS: overall survival; CEA: carcinoembryonic antigen; HER 2: Human epidermal receptor protein 2; ER: estrogen receptor; PR: progesterone receptor; EGFR: epidermal growth factor receptor; uPA: urokinase plasminogen activator; PAI-1: plasminogen activator inhibitor-1; MINDACT: Microarray In Node negative Disease may Avoid ChemoTherapy; TAILORx: Trial Assigning Individualized Options for Treatment [Rx] trial; CRC: colorectal cancer; AFP: alpha-fetoprotein; HCC: hepatocellular carcinoma; MSI: microsatellite instability; HCG: human chorionic gonadotropin; AUC: areas under the curve; SCC-Ag: squamous cell carcinoma antigen; PSA: prostate-specific antigen; NSCLC: non small cell lung cancer; NSE: neuron-specific enolase; NETs: neuroendocrine tumors; SCLC: small cell lung cancer; CgA: chromogranin A

Key Words: Tumor markers, Neoplasm, Clinical significance, Breast tumor markers, Gastrointestinal tumor markers, Gynecological tumor markers, Prostate tumor markers, Genetic markers, Review

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