Circulating Tumor Markers for Breast Cancer Management

14

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Abstract

Many patients with early stage cancers will go on to develop metastases. Blood based tests for circulating tumor markers can provide an invaluable minimally invasive method for assessing tumor and monitoring patients. Several markers are purported to be available for this purpose. These include some newer biomarkers such as tissue polypeptide antigens and serum autoantibodies against tumor associated antigens. In this chapter, we critically evaluate the available markers and describe their advantages and more importantly their limitations. A thorough review of the data available for these biomarkers leads us to conclude that sufficient evidence exists for the use of CEA, CA15-3, CA27.29 in metastatic breast cancers. However, none of the biomarkers are suitable for routine use in patients with early stage breast cancer. Novel blood based-biomarkers are urgently required to monitor patients with early stage breast cancer and predict the long-term outcomes.

Keywords

Circulating blood biomarkers · CEA · CA15-3 · CA27.29

14.1 Introduction

Breast cancer is the most common solid tumor in women, with a lifetime risk of 1 in 8, if a woman lives to age 80. While in general, breast cancer is a very treatable disease, with 75–80 % cure rates, it accounted for an estimated 40,356 deaths in 2014 in the United States alone and approximately 400,000 deaths worldwide (Porter 2008; Kamangar et al. 2006). Improvement in survival rates have been seen over the last few decades and these are largely attributed to earlier detection and

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S. Badve and Y. Gökmen-Polar (eds.), *Molecular Pathology of Breast Cancer*, DOI 10.1007/978-3-319-41761-5_14

improvements in treatment of micro-metastases that have spread to distant organs. These achievements are the result of better screening technologies and a better understanding of the underlying molecular makeup of the disease.

Improvement in treatment of cancer is best achieved when the disease is well understood from a biological perspective. This approach has been successful in breast cancer where tumor tissue 'biomarkers' are used to classify the disease. The 'molecular classification' allows the identification of relevant targets that are most likely to eradicate micro metastatic disease in early stages of breast cancer. In addition to tissue biomarkers, the disease can be assessed by circulating markers that are typically proteins, nucleic acids and cellular fragments that are shed by the cells and represent the underlying biology of the tumor. The detection of these circulating biomarkers is a significant challenge as they are much less abundant in blood and require special techniques to measure them. While, they represent 'surrogate markers' of the tumor tissue, there are additional challenges in distinguishing these markers from those of the host, as many of them are also seen in other conditions such as inflammatory diseases and benign causes of organ dysfunction. Typically, circulating markers from cancer are more abundant than those of normal processes, although the lines become blurry when small amounts of tumor are present in the body. The applications of these markers range from early detection, to diagnosis, to treatment and the following review will focus on these areas, in the context of each marker discussed. The markers included in this chapter are serum or plasma-based markers and nucleic acid and cellular components are discussed elsewhere.

14.2 Traditional Tumor Markers

Several proteins have been classically associated with breast tumors and also detected in the circulation. Traditionally, used tumor markers include carcinoembryonic antigen (CEA), soluble mucin1 protein or MUC 1 protein (CA27.29 or CA15.3), and the ectodomain (ECD) of the human epidermal growth factor receptor (serum HER2). In addition, autoantibodies that are tumor-specific are detectable in plasma and serum and are thought to be part of the host reaction to the presence of tumor tissue. These glycoproteins are thought to be secreted by tumor cells or the cells in the tumor microenvironment and can be detected in the peripheral blood with immunoassays.

14.2.1 Carcinoembryonic Antigen (CEA)

CEA is a glycoprotein, attached to the membrane by a GPI (glycosyl phosphatidyl inositol) anchor and is involved in adhesion to the extracellular matrix and plays an important role in cancer growth, invasion and metastasis (Blumenthal et al. 2005). It is a normal constituent of mucus that is secreted into the lumen by the glandular epithelial cells. With disruption of the normal tissue architecture, CEA is released into the vascular and lymphatic system. It is thought that the release of CEA into the extracellular matrix is due to GPI anchor cleavage catalysis by GPI specific phospholipase D type enzyme in in vitro experiments, but its mechanism of release is still under study.

CEA has been evaluated as a diagnostic/screening test, a prognostic marker and to monitor breast cancer during therapy. CEA has not been found to be useful for screening at the population level, as it is not sensitive or specific enough to differentiate between benign breast disease and breast cancer (Rimsten et al. 1979).

CEA is more promising in the setting of prognosis, as it is clearly associated with important outcomes and has been found to be reflective of disease burden. In a multivariate analysis, breast cancer patients undergoing surgery with elevated pre-operative levels of CEA had worse prognosis and higher risk of relapse after therapy (Gaglia et al. 1988). In early or localized breast cancer, CEA levels were noted to be similar before and after mastectomy, however, increases in levels after mastectomy are associated with increased recurrence rate (Wang et al. 1975). In patients with metastatic disease,

approximately 50–60 % of the patients have elevated CEA levels (Hogan-Ryan et al. 1980; Gray 1984; Tormey and Waalkes 1978; Veronesi et al. 1982). Furthermore, increases in CEA levels have been noted in cancer with metastasis to lymph nodes and distant organs (Laessig et al. 2007). While there is clearly a relationship between the detection and level of CEA and prognosis in early stage patients, there is no evidence that adding or changing therapy alters this prognosis. This concept, 'clinical utility', needs to be established in order for the biomarker to be recommended for clinical use. As a result, ASCO 2007 guidelines do not recommend CEA for determining prognosis among breast cancer patients, since clinical utility has not been established (Harris et al. 2007).

Perhaps the most useful setting to date for the use of CEA is in monitoring metastatic disease. Studies have shown that, among advanced breast cancer patients receiving hormonal therapy and chemotherapy, a drop in CEA levels correlates with response to therapy. Tormey et al., found that CEA levels >5 ng/ml pre therapy were associated withpoor response or early failure of chemotherapy (Tormey and Waalkes 1978). However, monitoring CEA levels does not meet the guideline requirement for clinical utility as it does not alter the prognosis when used for monitoring. Having said that, CEA and other similar markers may aid in therapy decision-making, in conjunction with other features of the clinical scenario. As a result, they are sanctioned by the ASCO Tumor Marker Guidelines Panel 2015 as reasonable adjuncts to physical examination and radiographic tests in the metastatic setting (Van Poznak et al. 2015).

14.2.2 Mucin 1 or MUC1 (CA15.3/CA27.29)

MUC1, a transmembrane glycoprotein (Fig. 14.1), is involved in oncogenesis by promotion of tyrosine kinase signaling, loss of epithelial cell polarity and constitutive activation of growth and survival pathways (Ren et al. 2006; Rajabi et al. 2014). In breast adenocarcinoma, MUC1 is overexpressed and under glycosylated resulting in loss of architectural demarcation between the apical and basolateral membrane in the cell. The most widely studied biomarkers are the soluble form of MUC1 (CA15.3), and mucin like associated antigen (MCA or CA27.29).

Similar to CEA, CA27.29 and CA15.3 have not been found to be adequately sensitive to be used for diagnosis. However, CA15.3 is found in the circulation of 10–15 % of stage I, 20–25 % and 30–45 % in stage II and stage III breast cancers, respectively (Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology 1996). Its usefulness is limited as marker in early disease but can be used in advanced stages for disease monitoring.

In early stage disease, elevated levels of CA15.3 are associated with worse outcome (McLaughlin et al. 2000; Gion et al. 2002). In addition, the prognostic impact of CA15.3 is independent of the tumor size and lymph node status (Tampellini et al. 2006; Gray 1984). However, as with CEA, there is no evidence that measuring these markers at diagnosis would not influence treatment decisions in a way that affects patient outcomes.

CA15.3 has been used in follow-up of breast cancer patients after the diagnosis and treatment of early stage disease. While measurement of the marker can provide a lead-time of 5–6 months for the detection of recurrent/metastatic disease in some women (Ren et al. 2006; Rajabi et al. 2014), there is no evidence that early intervention based on this lead-time improves outcomes or quality of life (Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology 1996). This is likely due to the fact that macrometastases that are detected at the time of recurrence are not curable with current treatment strategies and therefore finding these recurrences a few months earlier does not influence overall survival. This underlies the issue of sensitivity of many markers that makes them inadequate to detect micrometastases

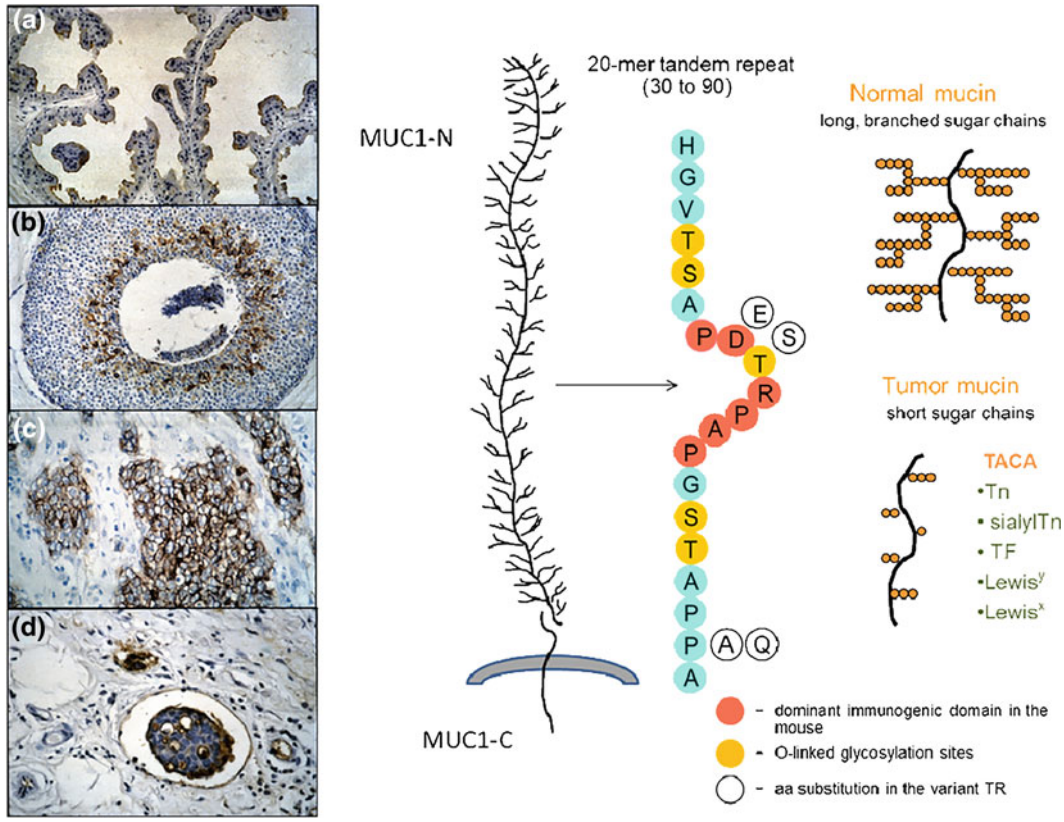

Fig. 14.1 MUC1 Molecule. To the right of the figure we have MUC1 molecule, the variant and nonvariant tandem repeat that form the major part of MUC1-N, and its glycosylation in normal and tumor associated mucin. To the left of the figure immunohistochemistry showing the expression of underglycosylated MUC1 in (**a**) apocrine metaplasia of the breast (**b**) ductal carcinoma in situ of the breast (**c**) invasive ductal adenocarcinoma of the breast (**d**) capillary with tumor embolus from an invasive ductal adenocarcinoma of the breast

at a curable stage. As with CEA, the MUC-1, CA15.3 and CA27.29 markers are not recommended for follow-up of early stage patients (Harris et al. 2007).

There have been a number of studies of CA15.3 in the metastatic setting. In an anthracycline based-phase II and III trial, median survival and clinical progression correlated with CA15.3 levels (McLaughlin et al. 2000). However, concordance with disease response was inconsistent. It has also been suggested that CA15.3 is useful for monitoring unevaluable disease such as pleural effusions, ascites, lytic and sclerotic bone disease, which are present in around one third of metastatic patients.

Studies utilizing CA15.3 and CA27.29 for monitoring patients have shown mixed results. (D'Alessandro et al. 2001; De La Lande et al. 2002; Guadagni et al. 2001; Kokko et al. 2002; Nicolini et al. 2006), and there are no randomized prospective clinical trials that determine the clinical utility of monitoring patients with metastatic disease. As a result, ASCO 2015 guidelines state that CA15.3 and CA27.29 should only be used in conjunction with other modalities like history, examination, and imaging to monitor treatment response in patients with metastatic disease (Harris et al. 2007; Van Poznak et al. 2015).

14.2.3 HER2/Neu Oncogene

The HER2 gene is located in chromosome 17q11-12 which encodes for transmembrane

receptor protein with tyrosine kinase activity. The overexpression of HER2 protein is detected in 15–30 % of breast cancer patients and has traditionally correlated with poorer outcomes. The extracellular domain (ECD) of HER2 is detectable in the serum and has been proposed as a surrogate for tissue levels of HER2 to predict early relapse or response to therapy. Like the other circulating markers, HER2 also lacks sensitivity and specificity for early detection and is not recommended for use in that setting.

Circulating HER2 levels have been studied in both the early and advanced stage settings of breast cancer and are consistently associated with a worse prognosis (Lee et al. 2016; Leitzel et al. 1995; Yamauchi et al. 1997; Volas et al. 1996). Serum HER2 levels are positively correlated with tumor size, tumor grade, and worse disease free survival in early stage disease (Burstein et al. 2003). In addition, studies have shown that ECD levels are associated with response to trastuzumab and hormonal therapy. In a study done by Ali et al. (2008), data was collected from 307 metastatic breast cancer patients from seven different institutions receiving trastuzumab based therapy. The serum samples were collected at baseline and at 30–120 days after initiation of trastuzumab. Sixty two percent patients had significant decline (>20 %) in serum HER2/neu and thirty eighty percent did not. The response rate was 57 % in patients with decline in serum HER2/neu compared to 28 % who did not. Patients with decline in HER2/neu levels had significantly longer time to disease progression (320 days vs. 180 days; $p < 0.001$), longer duration of response (369 days vs. 230 days; $p < 0.0001$) and longer overall survival (898 days vs. 593 days; $p < 0.018$). Based on this study data, patients with significant decrease in the HER2/neu levels >20 % were known to have decreased benefit from trastuzumab therapy (Ali et al. 2008). Given the complexity of calculating percentage declines and the variability around this number, HER2-ECD is not felt to be a practical measure for clinical use.

HER2-ECD has also been evaluated in metastatic patients in the context of trastuzumab with hormonal therapy. In a randomized controlled trial, patients with elevated HER2-ECD had lower response to letrozole versus tamoxifen. Serial measurement of HER2-ECD levels in these two groups of patients showed that patients with elevated HER2-ECD had overall lower response rates and had no advantage of letrozole over tamoxifen (Lipton et al. 2002). This suggests that this marker might be used to determine which patients are unlikely to respond to the combination of trastuzumab and any hormonal therapy and would be better served by a chemotherapy-based HER therapy combination.

Unfortunately, associations of HER2-ECD with therapy response are confounded by the fact that HER2-ECD levels are associated with increased tumor burden and a decrease in the half-life of trastuzumab antibody due to increase in the binding sites and accelerated clearance of immune complexes. These complex interactions make the use of HER2-ECD impractical and therefore it is not recommended in either the early or advanced disease setting (Harris et al. 2007).

14.3 Tumor Markers in Development: Protein Markers

Although, no biomarker is currently approved for early detection in clinical practice, emerging research on novel biomarkers for diagnosis, prognosis and response to treatment is underway and many promising markers are under development.

14.3.1 Tissue Polypeptide Antigens (TPA)

TPA is a complex polypeptide filament made up of cytokeratin 8, 18 and 19 produced mainly during the late S and G2 phase of the cell cycle. TPA can be elevated in benign conditions like renal failure, liver failure, pregnancy, diabetes mellitus (Tramonti et al. 2000), as well as a number of cancers, limiting its utility as a biomarker for early detection or diagnosis.

In the advanced stage setting, serum TPA levels were shown to be elevated in advanced cancers

(stage III and IV) patients, compared to localized breast cancer patients (Al-Youzbaki et al. 2014; Sliwowska et al. 2006). It was also shown that TPA levels are lower in breast cancer patients who received chemotherapy compared to patients who did not suggesting that it is associated with a worse prognosis as the patients who receive chemotherapy tend to have a higher disease burden and worse clinical features (Al-Youzbaki et al. 2014).

Tissue polypeptide–specific antigen (TPS) is a peptide epitope of cytokeratin 18 that can be detected in the serum (Bonfrer et al. 1994; Rydlander et al. 1996; D'Alessandro et al. 2001). As such it is thought to be a more specific serum marker than TPA and has been evaluated in several disease contexts. TPS has been found to be associated with higher tumor grade, and early stage patients with elevated tumor TPS levels have a higher risk of recurrence (O'Hanlon et al. 1996). A number of studies have suggested the utility of TPS as a prognostic marker. There are conflicting results in the literature regarding value of TPS marker in breast cancer (Given et al. 2000). On the contrary, TPS levels are known to be elevated in loco regional recurrence and significantly elevated to greater extent in metastatic diseases predicting different stages of the disease (O'Hanlon et al. 1996). Patients with elevated levels during follow up were likely to experience disease progression on further follow up. When compared to CEA or CA 15-3, TPS indicates proliferative activity, which is one of the most important phenotypic characteristics of tumor aggressiveness and is thus more beneficial as prognostic marker than serum markers as mentioned earlier (Bodenmuller et al. 1994; Weber et al. 1984; Hwa et al. 2008). Some studies have found that elevated pre-operative levels associated with poor disease free survival ($p < 0.001$) and low pre-treatment levels correlated with increased survival in advanced breast cancer patients (Ahn et al. 2013).

Several studies have suggested that TPS, particularly when combined with CA15.3, may be more specific and sensitive at predicting the likelihood of recurrence among breast cancer patients. However, larger scale studies and those aimed at clinical utility are needed to confirm these findings and support the recommendation of this marker in early stage disease. Thus, there are no recommendations as per ASCO Tumor Marker Guidelines for use of TPA or TPS in breast cancer (Harris et al. 2007).

TPS is known to be elevated in other inflammatory conditions like liver cirrhosis (van Dalen 1992) and in post-menopausal versus premenopausal women (Given et al. 2000), and is thus not specific enough to be recommended for screening or early detection.

14.3.2 Serum Autoantibodies Against Tumor Associated Antigens (TAA)

The 'Holy Grail' of serum tumor markers is to be able to use them for early detection, as this would reduce the need for non-specific radiographic screening of the entire population of women at risk for breast cancer, which currently is thought to be any woman over the age of 40 years. For many years, mammography has been the gold standard for screening that has been proven to have reduced mortality (Brooks 2009), but its sensitivity is reduced in patients with dense breasts (Brooks 2009). In addition, non-specific mammographic screening is thought to lead to over diagnosis and unnecessary treatments (Brooks 2009). Recently, there have been many serum tumor markers introduced like CEA, CA 15.3, estrogen receptor (ER) and progesterone receptor (PR), Circulating tumor cells (CTCs) which have been studied but unfortunately none of which have been approved for screening or early diagnosis of breast cancer due to lack of sensitivity and specificity of these circulating proteins. This prompted the evaluation of other serum markers that could improve these endpoints and led to intensive research on serum autoantibodies against tumor-associated antigens (Fig. 14.2).

Autoantibodies against p53 (Crawford et al. 1982), HER2 (Disis et al. 1997), MUC1 (von Mensdorff-Pouilly et al. 1996) and NY-ESO-1 (Stockert et al. 1998) were the first to be discovered in breast cancer patients. Studies have showed that serum collected prior to diagnosis, at

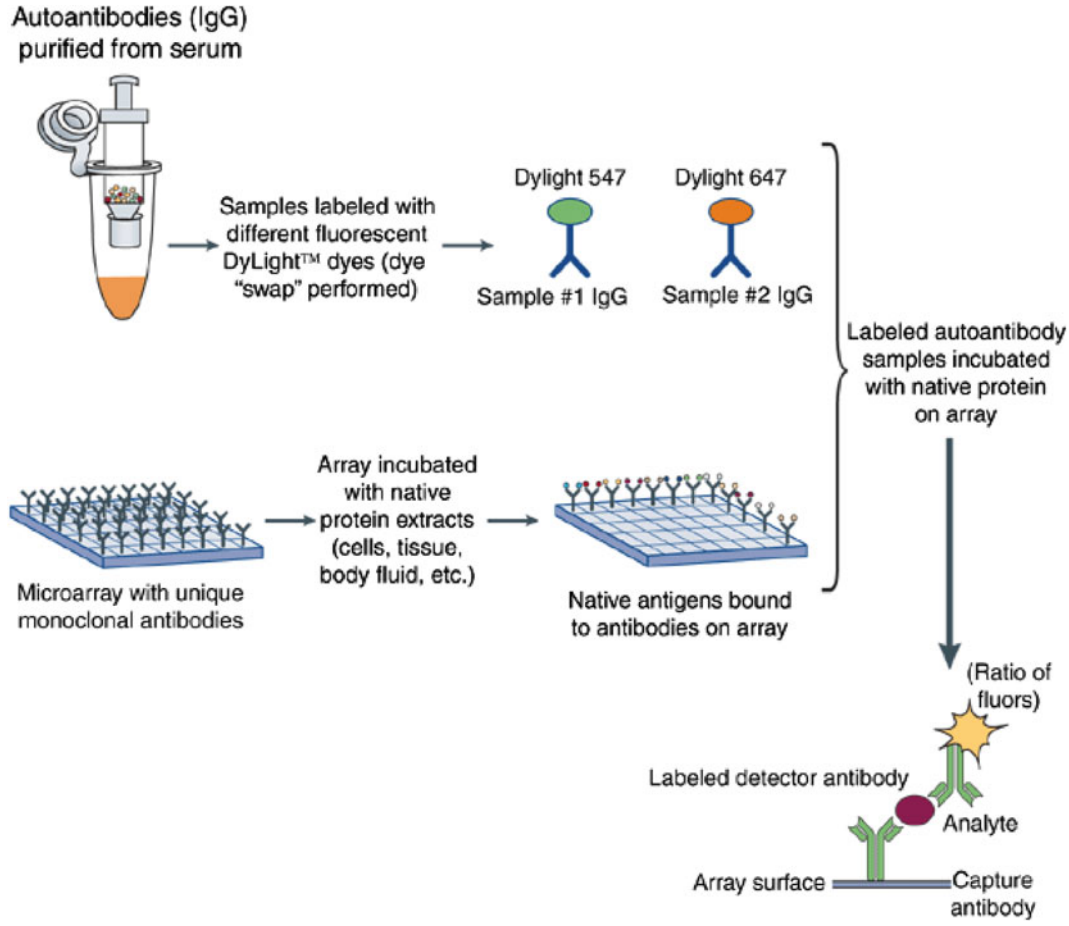

Fig. 14.2 ELISA method using antibody microarray to capture tumor associated antigens in the circulating blood. This figure depicts the use of autoantibodies purified from serum, are tagged with fluorescent dye. Native antigens in the circulating blood are bound to antibody on incubated array and result in fluorescent color from antigen antibody reaction

diagnosis, and during treatment showed that HER2 and p53 autoantibodies were significantly increased in samples from breast cancer patients. Elevated levels of HER2 and p53 autoantibodies can be detected in sera more than 150 days prior to diagnosis in breast cancer patients compared to controls (Lu et al. 2012). Recently, new autoantibodies such as SOX2 were found to be significantly elevated in patients with breast cancer (18.4 %) compared to healthy women (2.6 %) and (6.4 %) in patients with benign breast conditions. SOX2 antibodies were also associated with high tumor grade and positive nodal status. Other autoantibodies including p90/CIP2A show promising results (Sun et al. 2012) and are under investigation.

In patients with breast cancer, only 10–30 % of patients had a humoral response against a specific TAA, thought to be due to heterogeneous nature of the underlying biology (Tan et al. 2009). Looi et al. (2006) showed that p16 antibodies were relatively higher in nasopharyngeal cancer than in breast cancer. To confirm the specificity of p16 antibodies and to increase the frequency of antibody detection, a combination of TAA (p16, p53, and c-myc) was used. Antibodies to this antigen panel were found to be increased in frequency at $p < 0.01$. The combination of TAAs together increased the positive

antibody detection rate to a sensitivity of 44 %. Multiple studies have looked into TAA panels and the focus later has shifted to developing TAA panels to increase the sensitivity and specificity of the test (Fernandez Madrid 2005; Lu et al. 2008). This led to developing of different TAA panels with application of SEREX (autoantibodies like XI-A, p80, S6, RPA32 (Tomkiel et al. 2002) and NY-BR-I (Fernandez-Madrid et al. 2004; Brooks 2009; Levenson 2007; Jager et al. 2001) or SERPA (autoantibodies against RNA-binding protein regulatory subunit (RS), DJ-1 oncogene, glucose 6 phosphate dehydrogenase, heat shock 70-kDa protein-1 (HS71) and dihydrolipoamide dehydrogenase (Le Naour et al. 2001; Fernandez Madrid 2005). The TAA panels also increase the sensitivity and specificity for primary breast cancer and for ductal carcinoma in situ (Chapman et al. 2007) which can help in early diagnosis and can aid along with mammography for screening breast cancer.

Mammography has been shown to decrease the breast cancer mortality rates. The relative risk reduction is only 23 % and has recall rate for additional testing is 5–10 % in whom cancer would be detected. In women undergoing screening mammography, approximately 4 to 9 % have false positive test. There is clinical need for additional tests to aid in diagnosis of breast cancer, particularly in young patients under the age of 50 years in whom mammography is less sensitive (Levenson 2007). Chapman et al. investigated the use of autoantibodies to p53, c-myc, and HER2, NY-ESO-1, BRCA2 and MUC1 antigens by using the enzyme linked immunosorbent assay (Chapman et al. 2007). It was shown that autoantibodies were elevated repeatedly for one of six antigens in 64 % of primary breast cancer patients and 45 % of patients with ductal carcinoma in situ with 85 % specificity. Individual assay specificity for each antigen varied from 91 to 98 %. Hence these autoantibody assay against panel of antigens could be used with mammography for early detection of primary breast cancer especially in young women at risk. Due to heterogeneity of breast cancer and our limited understanding about autoantibodies against TAA, we need more definitive studies before tthey can be used in clinical practice.

14.4 Challenges in Utility of Circulating Tumor Markers

Multiple studies have shown the potential for utilization of circulating biomarkers for the clinical care of breast cancer from screening and diagnosis, to prognosis and treatment monitoring. However, only a few of these markers have successfully transitioned to routine clinical use. This section addresses some of the issues surrounding these challenges.

14.4.1 Cost Effectiveness

Health care costs are continually rising and becoming an increasing concern, particularly in the United States. Adding more tests to the treatment of a patient may increase costs and offers only limited benefits. A SEER-Medicare database analysis from 2001 to 2007 of the early breast cancer survivor patients evaluated the tumor marker tests for CEA, CA 15-3, CA 27.29 and health care claims through the billing codes and found that 42 % had received these tumor marker test within 2 years of diagnosis and the utilization increased over time from 38 % in 2001 to 46 % in 2007 (Ramsey et al. 2015). They found that the total cost of care for those patients with one test performed was 29 % higher than those not tested, often due to higher rates of advanced imaging (Ramsey et al. 2015). Given the financial constraints of current medical system, it is important to consider the benefit of a test before recommending routine use.

14.4.2 Poor Specificity

Certain tumor markers are also known to be de-regulated in other benign conditions. For example, like CA15.3 is elevated in chronic hepatitis, liver cirrhosis, hypothyroidism, and

sarcoidosis. Therefore, the utility of these biomarkers for early detection of breast cancer is low.

Further, paradoxically there can be increase in tumor markers concentration after commencement of chemotherapy possibly secondary to tumor cell necrosis. For example, Hayes et al. reported that there could be a spike in the CEA or CA 15-3 in 7 of 16 patients undergoing chemotherapy (Tondini et al. 1988). Therefore, many biomarkers used for monitoring ›treatment response need to be carefully defined as to when and how they are useful.

14.4.3 Lack of Reproducibility

Unfortunately, many promising new biomarkers that are reported in the literature fail to replicate in subsequent studies. For example, although circulating miRNAs were thought to hold great potential for breast cancer early detection, a review showed that the positive findings from these studies overlapped less than would be expected by chance (Tondini et al. 1988). Many studies of circulating biomarkers are not done in a rigorous manner and are done ad hoc with samples that are readily available. For example, using samples from a case-control design study to analyze a biomarker for early detection. Then, when the biomarker is tested in a sample of screening-eligible women in a prospective manner, the test does not replicate, as it is unclear how levels of these biomarkers change post-biopsy. Research networks, such as the Early Detection Research Network (EDRN) have been developed to help facilitate access to more appropriate samples.

Further, studies are often inconsistent in their protocols for collection and quantification of the biomarker. Some biomarkers, particularly many of these emerging cell-free markers, may be sensitive to time, temperature or processing. It is important for researchers from groups to collaborate and design strong biomarker studies with a number of independent replication sets.

14.5 Conclusion

Future direction towards identifying new tumor markers or new use of old tumor markers are essential. Many early stage breast cancer patients that are being treated surgically for cure, are prone to develop metastatic disease. We have insufficient data to recommend tumor markers like CEA, CA 15-3 and CA27.29 for diagnosis or monitoring of early stage disease but they can be used as adjunctive for monitoring the response to treatment in the metastatic setting. It is important that the clinicians are aware of sensitivities, specificities and limitation of each tumor marker before its use. In the recent past, investigators have focused on identifying new autoantibodies against tumor specific antigens and their role in breast cancer management. Many of these markers are still under study and have shown some promising results. It is crucial that we identify more of these tumor markers and explore their clinical applications. When new markers are identified, it is essential that we address the reliability and clinical utility of each marker. Only in this way, can we make progress in the management of breast cancer and improve outcomes for our patients.

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