SPANDIDOS PUBLICATIONS

Predicting response to neoadjuvant chemotherapy in breast cancer: Molecular imaging, systemic biomarkers and the cancer metabolome (Review)

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Abstract. The ability to predict the response to neoadjuvant chemotherapy (NAC) prior to or shortly after commencing treatment, in women with large or locally advanced breast cancers, would not only prevent patients from experiencing unnecessary drug morbidity but also reduce the high cost associated with drug usage and utilisation of resources with NAC. Ability to estimate residual cancer volume after NAC is of clinical relevance to subsequent therapeutic surgical options. Various approaches, using conventional histopathological characteristics and imaging modalities to evaluate and predict the response to NAC, have not been able to provide accurate and reliable data. Novel biomolecular imaging, new biomarkers and recent cancer genomic and proteomic profiling, introduced into clinical practice, have produced preliminary promising results. We describe and discuss these molecular characteristics and approaches and their applications to NAC in breast cancer management.

Contents

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1. Introduction

Neoadjuvant chemotherapy (NAC) is being used more frequently to manage large or locally advanced breast cancers (LABCs). The advantages of NAC are that not only may the tumour be downstaged (thereby, resulting in breast conserving surgery in some patients) but, more importantly, to possibly reduce the micrometastatic tumour load (1,2). Additionally, NAC can provide an opportunity to assess the likely outcome in any subsequent adjuvant therapeutic setting (3). The optimal chemotherapeutic regimen for NAC treatment in breast cancer is a combination of adriamycin and cyclophosphamide, followed sequentially by a taxane, which produces the best clinical response rates (60-90%) (3). A complete pathological response (a surrogate marker for long-term overall survival), unfortunately, is still <30% (3-5). However, these and other chemotherapeutic agents are associated with significant morbidity, are expensive and utilise resources. It would be advantageous if it were possible to identify patients who are most likely to benefit from NAC before or shortly after commencing the treatment. In the past, there have been no accurate and reliable indicators or markers to predict response to the drugs before commencing NAC. Various biotechnologies, including both imaging and biomolecular platforms, have been investigated in order to find novel biomarkers or tests to predict responses to NAC. Here, we review and discuss recent biotechnological innovations and developments that have shown promise and possible application in clinical practice (Fig. 1).

2. Molecular imaging studies

Significant developments in molecular imaging during the last two decades have greatly enhanced the accuracy of diagnosing breast cancer, and show promise in predicting the response to drug therapy. NAC induces cancer cell death by promoting apoptosis and cell necrosis. As a result, tumour volume is reduced and there is variable tumour shrinkage in the breast. However, in some cases, the connective tissue stromal component of the tumour may persist and the destroyed cancer cells can be replaced by a hyaline amorphous scar, both of which can result in the misinterpretation of the residual

tumour mass. Thus, imaging based on anatomical features and physical coordinates, including mammography and ultrasonography, cannot be used to predict accurately and reproducibly the response to NAC (6,7). However, the accuracy can be improved to some extent by combining these modalities together in assessing the pathological response to NAC (8,9). This approach is cost-effective and readily available in most hospitals. Recent novel imaging technologies introduced into clinical practice to predict responses to NAC have employed the biomolecular properties of cancer cells (instead of anatomical or structural characteristics alone) to both evaluate and predict the responses to NAC. Though the preliminary results look promising, most are limited by the small number of participants recruited into the studies and lack of randomised controlled trial data.

Magnetic resonance imaging (MRI). MRI utilises powerful magnets to alter the orientation of the protons in the water molecules of the cells, thereby, producing radio wave pulses which are converted into visual images. Dynamic contrast-enhanced MR imaging with gadolinium-based contrast intravenous injections enables some of the functional effects of tumour vascularity to be studied *in vivo*. MRI has a high sensitivity but relatively poor specificity in the detection of primary breast cancers and tumour recurrence (10). MRI, however, can effectively distinguish between recurrence and fibrosis or scar tissue, and has been shown in recent studies to demonstrate a high accuracy rate for the prediction of residual tumour following NAC (11-13). MR mammography with contrast enhancement has been documented to be correlated with various angiogenic markers [microvascular density, vascular endothelial growth factor (VEGF) expresion] in breast cancers and has been shown to predict pathological responses to NAC accurately after two cycles of chemotherapy (14,15). This is possibly due to a decrease of tumour microvascular permeability and blood flow (transfer constant) after successful chemotherapy treatment. However, there are limitations of the technique due to cost and accessibility, and further results from larger trials are necessary to confirm these promising findings.

Scintimammography. Another imaging modality that is being used more frequently in breast cancer diagnosis is 99mTc-Sestamibi (MIBI) scanning. The increased uptake of MIBI in malignant tissue is probably due to enhancement of angiogenesis and the oxidative metabolism of malignant cells (16). MIBI is a transport substrate for P-glycoprotein (Pgp), a multi-drug resistance-associated glycoprotein, found to be overexpressed in the cell membranes of chemoresistant cancers (17). Retention of MIBI, therefore, has been postulated to correlate with enhanced chemosensitivity and, thus, useful in prediction of chemoresponsiveness (18-21). However, a single pre-treatment scan is not sensitive enough and serial scanning should be performed (22). There has been, so far, limited supporting data due to lack of a suitable randomised trial. MIBI has also been used to assess residual tumour volume following NAC, but was unable to predict this with accuracy. This failure is postulated to be due to the enhanced expression of Pgp in drug resistant cells and the failure of MIBI to be retained by such cells (19,22).

Positron emission tomography (PET). PET is a non-invasive, functional molecular imaging modality that detects positron-emitting radiopharmaceuticals linked to metabolically active molecules introduced into the body. It has recently been used to predict responses to NAC in breast cancer. Malignant cells are estimated to have a five times higher uptake of glucose than normal cells, due to more prominent expression of the glucose transporter, Glut-1 (23). A glucose analogue, 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}FDG), is the most commonly used positron-emitting tracer in oncological imaging. In malignant disease, there is incomplete intracellular degradation of ^{18}FDG in cancer cells. Thus, detection of ^{18}FDG accumulation following NAC can reliably detect residual tumour volume. The limitation of ^{18}FDG-PET are partial volume effects and some varying metabolic activity effects depending on tumour type (24).

Smith *et al* (25) conducted serial ^{18}FDG-PET scanning in 31 breast cancer patients who received NAC and compared the reduction rate of ^{18}FDG uptake with both clinical and pathological responses. The results showed that the early mean reduction of ^{18}FDG uptake in cancers with a complete pathologic response was significantly higher than with less responsive tumours (25). Comparable results were confirmed by other reports (26-28). A single scan was not always reliable but a high degree of accuracy was seen with two scans (29). Serial PET scans, however, have limitations in terms of cost, availability and doses of radionuclide exposure.

Diffuse optical spectroscopy (DOS) and imaging (DOI). DOS is a novel non-invasive technique currently being evaluated in the detection of tumours. The near-infrared (NIR) absorption spectra is measured with DOS. The absorption spectra determines the tissue concentration of oxygenated haemoglobin (ctO_2Hb) and deoxygenated haemoglobin (ctHHb), water (ctH_2O) and lipid (30). The results from DOS/DOI in predicting the response to NAC are preliminary. In a small pilot study using DOS/DOI in 11 patients with breast cancer, the response could be predicted when changes of ctHHb and ctH_2O were summated. In patients receiving adriamycin responses were documented with a sensitivity and specificity of 100%, one week after infusion of the drug (30). The technique is non-invasive, easy to use and requires no radioactive isotopes. However, larger and further studies are required to establish its application in clinical practice.

3. Serum tumour biomarkers

Many serum tumour biomarkers have been proposed in breast cancer, including the MUC-1 antigen (CA 15.3), the onco-foetal protein carcinoembryonic antigen (CEA), the oncoprotein HER-2/neu and the cytokeratin tissue polypeptide specific antigen (TPS). Amongst these, CA 15.3 and CEA are the most widely used in clinical practice. High levels of these serum tumour biomarkers have been correlated with poor survival and are a measure of metastatic tumour load, but their value in screening and the early diagnosis or recurrence of breast cancer are problematic due to lack of tumour specificity and multi-organ distribution (31). The markers are best used in combination, and serially, to detect the recurrence of both local and distant metastases after treatment.

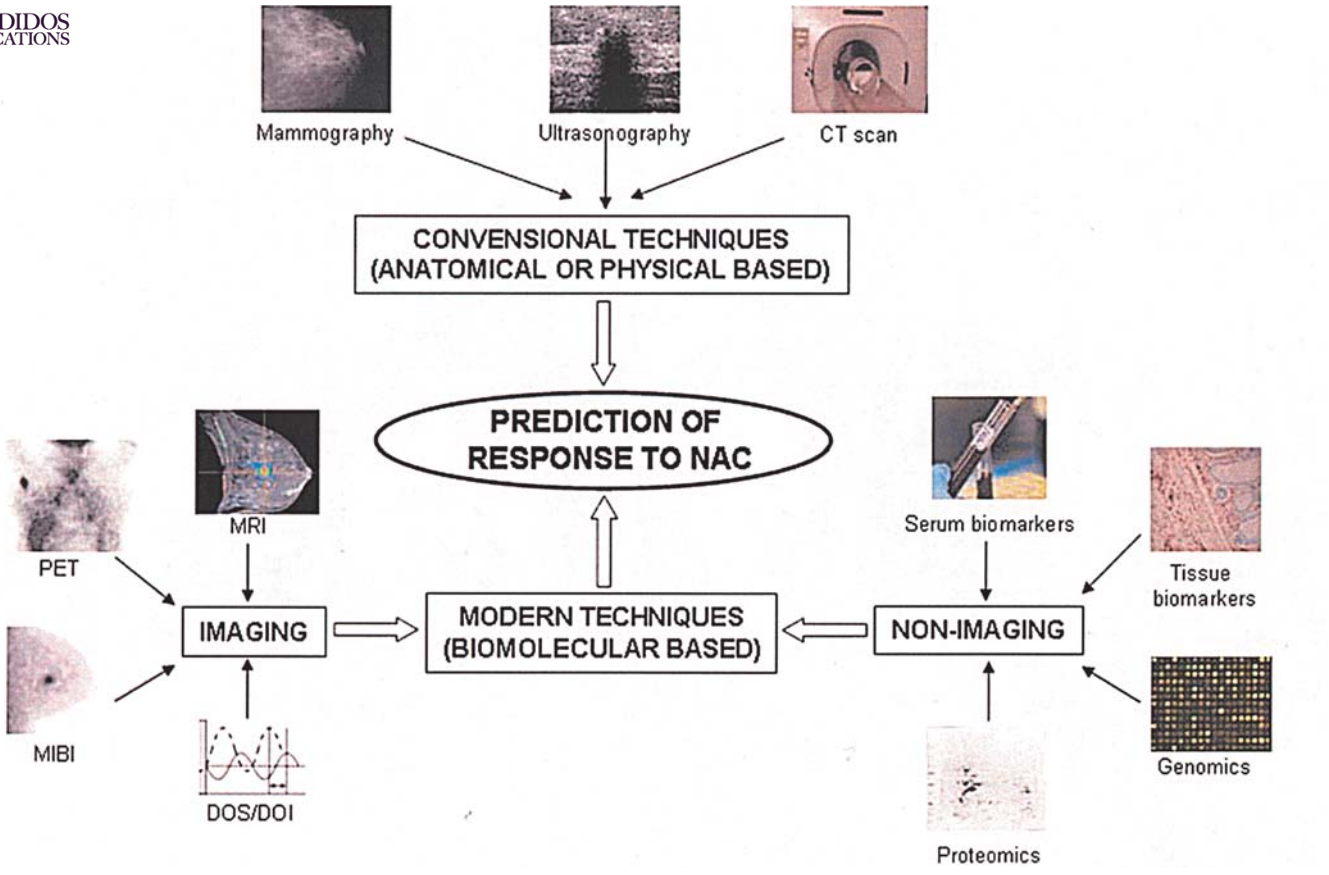

Figure 1. The illustration summarises the modalities outlined in the article to predict response to NAC. Novel technologies using molecular characteristics of cancer cells are currently being investigated; these include both molecular imaging and non-imaging profiles. Molecular-based techniques are expected to replace conventional anatomical-based techniques to predict response to NAC in breast cancer.

In two small retrospective studies, the prediction of NAC treatment was promising. In a study monitoring serial serum CA 15.3 and TPS in 39 women who underwent NAC, correlation with the clinical response to NAC was 66.7% (32). In a further study in 75 women with breast cancer, high levels of pre-treatment CA 15.3 and its fall following NAC predicted both clinical and pathological responses to NAC (33). A larger retrospective study in 348 women with advanced breast cancer showed a good association between the reduction in elevated CA 15.3 and CEA and response to treatment (34).

The use of these tumour biomarkers is well established in detection of recurrence or metastatic disease. However, the incidence of elevated markers in women undergoing NAC is low, even in patients with advanced disease (20-30% for CA 15.3) (32-34). In those patients who have normal pre-treatment levels, the predictive value is unhelpful (34).

4. Tissue biomarkers

Over the years, many tissue biomarkers have been used in breast cancer management. These include hormone receptor status, HER-2/neu expression, DNA ploidy and S-phase, and detection of Ki-67. However, these bio-markers have been used mainly for general prognostic assessment and suitability for specific drug therapies; accuracy as predictors of NAC is ill-defined (35).

Various other biomarkers have been extensively researched and show potential as predictors of response to chemotherapy in breast cancer. These markers include the multi-drug-resistant P-glycoprotein, the oncogene C-*myc*, cell cycle and apoptotic-related factors (*p21, p53, Bcl*), the cell adhesion molecule E-cadherin and VEGFs. For further information, the reader is referred to recent publications (35-37). From the data published, so far, none of these tumour tissue bio-markers are either sensitive or reliable enough to use in the clinical setting. Some show promising results but need to be further validated.

5. Gene expression profiling

This emerging biotechnology has been used recently in various aspects of breast cancer studies, including early diagnosis, prediction of survival and prediction of response to drug therapy. Factors that determine a good response to NAC are complex, multifactorial and depend on multiple genes and proteins. Therefore, multiple rather than single gene markers need to be used to predict likely responses to NAC (38). Sotiriou *et al* (39) successfully reported the value of pre-treatment fine needle aspiration sample gene expression profiling in predicting clinical response in women with breast cancer who underwent NAC (39). Chang *et al* (40) demonstrated the correlation between the expression of 92 genes (selected from 6849 genes following cDNA analysis)

and clinical response in 24 women who underwent NAC with docetaxel. The sensitivity and specificity in predicting the response to docetaxel in these 92-gene predictors was 85 and 90%, respectively (40). Other subsequent studies also showed the potential of this technology in predicting the response to NAC (38,41,42). However, the technology is complicated and expensive and the interpretation of the microarray results is also complex with interlaboratory variations. Gene microarray profiling appears to show promise in predicting response to NAC, but does require further study and validation.

6. Protein expression profiling

Proteomics is another molecular biotechnology that is being studied in cancer. The development of many proteomic platforms including MALDI, SELDI mass spectrometers, two-dimensional gel electrophoresis and laser captured microdissection have all improved the likelihood of their application in human tissue samples.

SELDI-TOF technology has been used successfully, and impressively, to diagnose early ovarian cancer, as well as breast cancer from human serum (43,44). However, the application of proteomics in human breast tissue samples requires further development. To the best of our knowledge, there is no publication to date regarding the use of proteomic profiling in predicting the outcome of NAC in human breast cancer. Data on *in vitro* breast cancer cell lines looks promising and is a good model to develop strategies in humans (45).

7. Conclusion

The assessment and prediction of response to NAC in women with LABCs is a major and continuing clinical challenge. Molecular imaging modalities of MRI, MIBI and PET scanning, and DOS/DOI, introduced over the past two decades have demonstrated great potential and preliminary satisfactory results. To date, data on MRI and PET (prediction of likely response to and residual disease following NAC) look promising but cost-effectiveness, ready availability of the technology and validation in larger numbers need to be further addressed.

Serum and tissue biomarkers hold some promise but sensitivity, specificity and accurate and consistent applicability in the clinic are lacking. Very recent gene expression profiling looks promising but requires further validation. Preliminary data on *in vitro* studies with proteomic profiling shows potential but there is a dearth of clinical data. It is very likely that further important developments in molecular imaging and a better understanding of the metabolome of the cancer cell in the near future will enhance our ability to selectively target specific drug combinations to produce complete pathological response rates, obviating unnecessary drug-related morbidity and minimising the extent of breast surgery. The 'blunderbuss' approach, currently used, will become a therapeutic relic of the past.

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