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# Serum CK18 as a Predictive Factor of Response to Chemotherapy in Locally Advanced and Metastatic Breast Cancer

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## Abstract

**Introduction: Breast cancer is the most common cancer in women and the second most frequent cause of cancer death. Several factors affect response to chemotherapy including nodal status, hormonal status and human epidermal growth factor receptor (Her-2). Aim of Study: The study is aiming at evaluating M30 antigen in serum of patients with locally advanced and metastatic breast cancer and establishing the relation between M30 level and response to chemotherapy. Patients and Methods: The study was performed at Al Bairouni University Hospital and the Faculty of Pharmacy (Damascus-Syria). We have included 60 patients with histologic confirmation of invasive ductal carcinoma of the breast treated with the combination (Docetaxel + Doxorubicin) with M30 levels to be evaluated before treatment and 24 hours after the first and third cycle. Results: M30 level increase in serum 24 hours after the 1st cycle correlated with different kinds of response in 39 patients (P value less than 0.03) with better results in those with Estrogen Receptors (ER) positive patients (P value 0.05). There was no correlation between Her-2 status and response (P value 0.3). Conclusion: M30 level in serum is a useful predictor marker of response to chemotherapy in both locally advanced and metastatic breast cancer.**

## Keywords

**CK18, Metastatic Breast Cancer, Response**

## 1. Introduction

Breast cancer remains the most common cancer in women and the second most frequent cause of cancer death

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[1]. There are a number of factors which determine the prognosis of disease and response to treatment. Prognostic factors are those which determine the outcome of disease in the absence of systemic treatment whereas predictive factors predict response to treatment [1]. Estrogen receptor (ER) and progesterone receptor (PR) expressions are the most important and useful predictive factors currently available. ER and PR are intracellular steroid hormone receptors which have received substantial attention since 1986. Measurable amounts of ER and PR are found in about 50% - 85% of patients with breast cancer. The frequency of positivity and the level of ER and PR increase with age, reaching their highest levels in postmenopausal women [2]. Apoptosis (programmed cell death) plays an important role in tissue homeostasis and development. It is regulated by a wide variety of survival signals as well as cellular mechanisms that are in charge of DNA integrity [3]. The apoptosis execution mechanism results in the development of characteristic morphological features such as nucleus and chromatin condensation, cell shrinkage and cytoplasmic blebs. The measure for quantification of apoptosis is the apoptotic index (AI). Many publications have proved that breast cancers with a high apoptosis index (programmed cell death) have a better prognosis compared with the same type of cancer with less or no apoptosis [4].

Although multiple genes are involved in apoptosis, the key mediators of the process are the Caspases. Caspases are Aspartate-specific Cysteine proteases, which cleave their substrates on the carboxyl side of the Aspartate residue and Cytokeratin 18 [5] [6]. Currently at least 14 different Caspases are known to exist, of which two thirds play a role in apoptosis. The Caspases involved in apoptosis can be divided into two main groups, the initiator Caspases (e.g., Caspases 8, 9, and 10) and the downstream effector Caspases (e.g., Caspases 2, 3, 6, and 7). It is the members of the latter group that degrade multiple cell proteins and are responsible for the morphological changes in apoptosis. Caspase 3 is the most widely studied of the effector Caspases. It plays a key role in both the death receptor pathway, initiated by Caspase 8, and the mitochondrial pathway, involving Caspase 9. In addition, several studies have shown that Caspase 3 activation is required for apoptosis induction in response to chemotherapeutic drugs e.g., Taxanes, 5-Fluorouracil, and Doxorubicin [7]-[10]. Cytokeratin 18 (CK 18) is a member of cytoskeletal protein family which is present in epithelial cells [11]. When apoptosis is induced, CK 18 is cleaved from Aspartate amino acids localized at position 238 and 396. Monoclonal antibody M30 recognizes the neoepitope of CK 18 formed after cleavage by Caspases. This newly-formed neoepitope can be regarded as a selective biomarker of apoptosis [12] [13]. Recently, it was reported that serum M30-antigen levels may also be a prognostic marker in some tumor types [14] [15]. In another study, M30-antigen was reported to be associated with the survival in advanced gastric carcinoma patients [16]. Some studies investigated Casepases and their role in breast cancer, some of which studied Caspase levels and expression in breast carcinoma at mRNA level, however, little work has been made to evaluate levels of Caspases in serum of patients under treatment; therefore, the aim of this study is to test the concentration of CK18 level in serum of patients with locally advanced and metastatic breast cancer, then to evaluate the relation between CK18 concentration (fold increase) and response to chemotherapy. In our study, we tried to show the importance of CK18 as a predictive marker of response on chemotherapy in both locally advanced and metastatic breast cancer.

## 2. Patients and Methods

### 2.1. Patients

The study is prospective, initiated in September 2012 and included 102 persons (21 disease free patients on follow up, 21 healthy volunteers and 60 patients diagnosed with locally advanced and metastatic invasive ductal carcinoma of the breast). 25 patients presented with hepatic metastasis, 22 with pulmonary metastasis, 3 with bone metastasis and 10 others with locally advanced disease. Age of patients was between 28 and 62 years (51 years in median), good performance status (0, 1 and 2), normal hepatic and renal functions and pathologic confirmation of disease. The study was performed at Breast cancer unit (al Bairouni university hospital) and the faculty of Pharmacy Labs in Damascus (SYRIA). Both locally advanced and metastatic group received the same combination chemotherapy (Docetaxel 75 mg/m$^2$ and Doxorubicin 75 mg/m$^2$) repeated every 21 days for 3 cycles then evaluated by CT-Scan and bone scan for those with metastatic disease and by clinical exam for those with locally advanced disease

### 2.2. Methods

Blood samples were taken from healthy cohort, disease-free then from patients before beginning of treatment,

after 24 hours after the first cycle and 24 hours after the completion of the third cycles. Samples were centrifuged and serum was collected and M30 antigen was tested by means of (ELISA). The test measures M30 concentration in serum and CK18 fragments due to K18Asp396 containing Caspase (M30 Apoptosense ELISA) obtained from PEVIVA (Sweden), Kit (ALX-850-270-KI01).

### 2.3. Biostatistics

Correlation between parameter was assessed by Spearman's test, while Mann-Whitney test was employed to compare between means. To assess the relation between types of response and M30 values after 24 hours of the first chemotherapy, Qui square was used. Statistical significance was assigned to P value less than 0.5.

## 3. Results

Of the 60 patients included in our study, the disease progressed in 21 patients (35%) with less than 0.5 fold increase in M30 concentration after 24 hours of the first cycle. 10 patients (17%) with M30 concentration fold increase between 0.5 - 0.9 showed stable disease while the remaining 25 patients (42%) showed partial response with M30 fold increase between 1 - 1.9. In the other hand, the 4 complete responders (6%) showed a fold increase between (2-5) 24 hours after the completion of the first cycle as illustrated in **Table 1**.

The mean concentration of M30 was 183 U/L in disease-free patients on follow up versus 182 U/L in healthy volunteers (21 persons each). Concentrations were compared between the two former groups using Mann-Whitney test, showed P value 0.9 with no difference in concentration between the two groups. The test was extended to compare M30 concentration between (healthy and followed-up patients) versus patients before treatment, showed no difference with P value of 0.69.

Interpretation showed a direct correlation between M30 concentration before treatment and 24 hours after the first cycle (P value 0.04), however, M30 increase was not significant after the completion of the third cycle (was not predicting of response) with P value of 0.6. Mean concentration of M30 before treatment, after the first cycle and after the third cycle gives a good indicator of response in both locally advanced and metastatic disease. For example, mean M30 concentration was elevated in patients with metastatic disease (50 patients) compared with those with locally advanced disease (10 patients) as shown in **Table 2**. To evaluate the statistical relation between M-30 fold increase (before and 24 hours after the first cycle) and response to treatment, Chi square was employed showing P value of 0.03.

Among the 60 patients, 35 patients had Estrogen receptors (ER) positive disease (58%), so disease progressed in 8.9% of patients with ER positive disease versus 26% of patients with ER negative disease reflecting a better prognosis in those with ER positive group with P value of 0.05.

Furthermore, M30 concentration at baseline was elevated in Human epidermal growth factor receptor positive patients (Her-2 +) (43 patients) compared with those with Her-2 negative patients with a significant P value of 0.018. However, Her-2 status was not a predictor factor of response (P value 0.3).

**Table 1.** The relation between M30 concentration and type of response.

| Number of patients | M30 fold increase | Type of response | Percentage |
|---|---|---|---|
| 21 | <0.5 | progression | 35 |
| 10 | 0.5 - 0.9 | Stable | 17 |
| 25 | 1 - 1.9 | Partial response | 42 |
| 4 | 2 - 5 | Complete response | 6 |

**Table 2.** The difference between M30 concentration between locally advanced and metastatic disease.

| Type of disease | | M30 baseline | M30 after 1st cycle | M30 after 3rd cycle |
|---|---|---|---|---|
| Locally advanced | Mean | 99.66 | 254.00 | 194 |
| | Number | 10 | 10 | 10 |
| | St-deviation | 34.32 | 137.61 | 127.79 |
| metastatic | Mean | 329.20 | 586.82 | 317.11 |
| | Number | 50 | 50 | 50 |
| | St-deviation | 298.49 | 472.59 | 314.80 |

## 4. Discussion

In our study, we measured serum M30 antigen levels in patients with both locally advanced and metastatic breast cancer to reveal the relation between M30 antigen levels and response to chemotherapy protocol (Docetaxel + Doxorubicin). In neoadjuvant and metastatic setting, we do not know which patient will respond better to chemotherapy. Response to chemotherapy is considered a good prognostic factor and may predict a long progression free survival period [17]. Our study showed that M30 antigen elevation especially 24 hours after the first cycle is the most important predictor of response on the short term, and there is a direct proportion between fold increase and degree of clinical and radiologic response.

Death of tumor cells generates detectable protein products in the patient's circulation, which may be used for cancer diagnostics and/or monitoring of therapy efficacy [18]. Apoptosis is a form of regulated cell death that is characterized by specific structural changes, mediated by proteases of the Caspase family [19]. The M30 antibody detects a Caspase-degraded product, CK18-Asp396 (also called M30-antigen), of the important

Cytoskeletal protein called Cytokeratin 18 of epithelial cells. Cytokeratin 18 is expressed by most carcinomas, including those of breast, prostate, lung and colon [13]. It has previously been shown that circulating M30-antigen levels increased in patients with various cancer types and, furthermore, it increased during chemotherapy. For instance, the Docetaxel treatment increased levels of M30-antigen in the serum of breast cancer patients, indicating apoptotic death of tumor cells, while the Cyclophosphamide/Epirubicin/5-fluorouracil treatment led to a heterogeneous response with regard to cell death mode [20]. In preclinical models, studies have shown an increase of apoptotic proteins 1 - 3 days after chemotherapy [21]-[23]. In our study and in a similar way, M30 antigen elevated 24 hours after the first cycle with a serum level decreasing over time to reach a nadir after the 3$^{rd}$ cycle which could be attributed to decrease in tumor volume and consequently apoptotic proteins in responders, however, low level of M30 after the first cycle accompanied with lower levels over time to reveal a chemoresistant cancer cells. This finding may help researchers and clinicians to best tailor the treatment of locally advanced and metastatic breast cancer and to predict the chemoresistant patients from the very beginning. But, if we find low levels after the first cycle, are we able to change chemotherapeutic protocol? It is still a hard question to answer so far, because other factors may affect response to chemotherapy such as ER status, Her-2 status and nodal status.

Regarding chemotherapy, Taxanes induce mitotic catastrophe, characterized by the occurrence of aberrant mitosis followed by cell division. Mitotic catastrophe is not a cell death mode, but will trigger cell death, either by apoptosis or by nonapoptotic mechanisms [24]-[26] and our study is supporting this concept through elevation of M30 in vivo after treatment with Docetaxel in combination with Doxorubicin. Therefore, we can conclude that M30 level in serum could be used as a predictive factor of response in patients with measurable disease breast cancer, however, further studies with other protocols are warranted to tailor our treatment in a better way.

## Disclosure

The authors declare no conflict of interest.

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