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Reliability of CA15-3 Tumor Marker in Monitoring Therapeutic Response in Different Molecular Subtypes of Metastatic Breast Cancer

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Abstract

Background: The prognostic impact of CA15-3 level in different molecular subtypes of metastatic breast cancer is not well elucidated yet; therefore, we conducted the present study to determine the reliability of CA15-3 tumor marker in terms of monitoring therapeutic response in different molecular subtypes of breast cancer.

Method: In this prospective study, we assessed the levels of CA15-3 in 83 patients with metastatic breast cancer assessable by RECIST, who were treated and followed up in Mosul Oncology hospital during 2017 and 2018. We evaluated the mode of changes of CA15-3 level after two cycles of systemic therapy (chemotherapy, endocrine therapy, or target therapy) and analyzed the relation between CA15-3 level changes and response to therapy in different molecular subtypes of breast cancer.

Results: Herein, CA15-3 level was more frequently elevated in Luminal subtypes of metastatic breast cancer compared with that in other subtypes. Additionally, the reduction in CA15-3 level after two cycles of systemic therapy was significantly correlated with the good response and longer progression-free survival.

Conclusion: The mode of change of CA15-3 level was closely correlated with the clinical therapeutic response and survival advantage rather than the pretreatment level of CA15-3 in metastatic breast cancer. This finding revealed equivalent quality of CA15-3 with medical imaging at lower cost. Therefore, measurement of CA15-3 level at regular intervals before and after starting systemic therapy could predicate the clinical response and replace imaging examination used routinely for monitoring the responses in patients with Luminal subtypes of metastatic breast cancer.

Keywords: Breast neoplasms, Subtypes, Tumor marker, CA15-3

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Introduction

Cancer of the breast is the second most prevalent cancer worldwide and the most common cancer among

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women.[1] It is one of the main causes of mortality throughout the world.[2] Owing to further medical progress in early diagnosis and treatment, its mortality rate has declined.[3] However, distant metastases are still observed in about 20%-30% of breast cancer patients,[4,5,6] leading to approximately 40,000 breast cancer mortality annually.[7] Metastatic breast cancer remains incurable in the majority of cases, and treatment only aims to improve the quality of life and survival; therefore, there are serious challenges now concerning a non-invasive reliable test for assessing the treatment response.[8] Radiological examination is a standard monitoring strategy that has been used widely for the assessment of treatment response. Nonetheless, more than 30% of metastatic lesions are not measurable by medical radiological test. Accordingly, identifying a more convenient marker is of particular importance for monitoring the treatment response and reducing the use of radiology for re-staging.[9] Serum tumor markers play important roles in many types of cancer regarding early diagnosis, prognosis determination, predicting response to special type of therapies, and the early detection of recurrence after surgical operation.[10]

A great deal of attention has been paid recently to the prognostic value of CA15-3 in breast cancer patients.[11] CA15-3 tumor marker is a mucin belonging to a big family of glycoproteins encoded by MUC 1 gene that are expressed on the surface of normal epithelial cell types, including those of the breast epithelial cells.[12]

It has been shown that most of the patients with metastatic breast cancer have high levels of CA15-3, while its level rarely increases in patients with early stage or localized cancer.[10] However, Canizares F. et al. reported that the measurement of CA15-3 level before surgery is significantly related to the outcome in patients with early breast cancer. In addition, patients who got high levels of CA15-3, have a significantly worse prognosis compared with those with low levels, in terms of the overall survival (OS) and disease-free survival; this may be due to a heavy burden of occult disease.[13]

Since breast cancer is a heterogeneous disease, histopathological classification alone will not satisfy variable clinical outcomes of the disease. Molecular classification is a mandatory way to predict prognosis and outcome. Recently, molecular classification, based on immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67 markers, has widely utilized as a surrogate for gene expression analysis and for providing both prognostic and therapeutic information.[14]

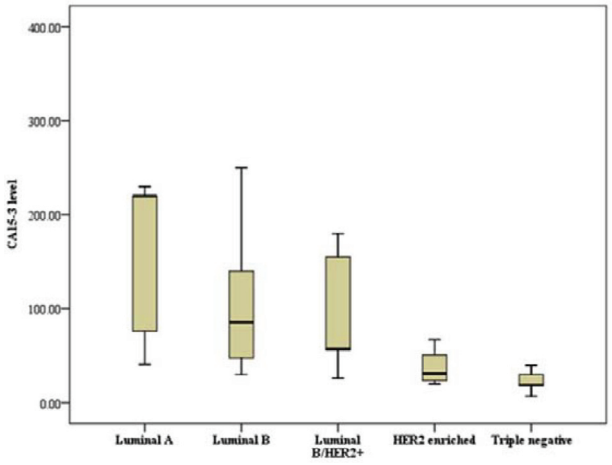

Figure 1. Boxplot of CA15-3 level in different molecular subtypes of breast cancer showed that CA15-3 elevation was more frequent in Luminal subtypes than in HER2 enriched and triple negative subtypes at the time of diagnosis of metastatic breast cancer.
HER2: Human epidermal growth factor receptor 2

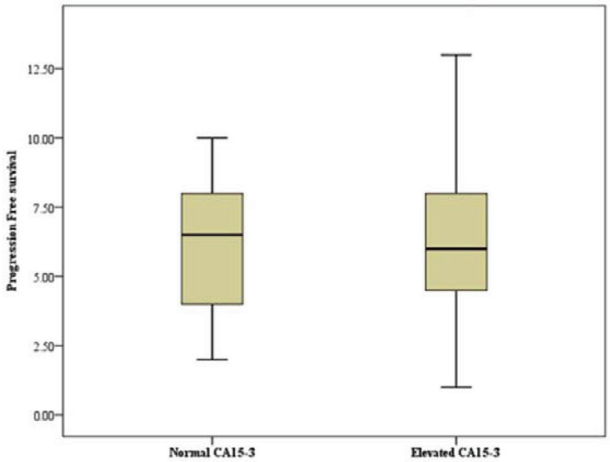

Figure 2. Boxplot of CA15-3 levels at the time of diagnosis of metastatic breast cancer showed no significant association between the level of CA15-3 at the time of metastasis and progression-free survival.

The prognostic impact of CA15-3 level in different molecular subtypes of metastatic breast cancer still remains unclear; thus, we conducted this prospective study to determine the reliability of using CA15-3 tumor marker for monitoring therapeutic response in different subtypes of breast cancer based on the recent refinement of the molecular classification done during the last Saint Gallon conference.[15] It recommended the use of Ki67>20% as a cut point for differentiating Luminal B/HER2 negative subtype from less aggressive tumor Luminal A subtype.

Patients and Method

In this prospective cohort study, we recruited 83 patients with metastatic breast cancer assessable by RECIST, who were treated and followed up in Mosul Oncology hospital during 2017 and 2018. Computed tomography (CT) scan, magnetic resonance imaging (MRI), and / or Positron emission tomography CT (PET / CT) scan were done for them to determine the extent of the disease and confirmation of the metastasis was carried out by true-cut biopsy from the metastatic lesions. Out of all the subjects, 51 presented de novo with stage IV disease and 32 had a history of breast cancer and presented with recurrent disease.

Appropriate ethical approval for this study was obtained from the Ethical Committee of College of Nursing/ University of Mosul (Code: 20/012019).

Molecular classification

The staining of the tissue samples, which has been fixed with formalin and embedded with paraffin, was done via immunohistochemical markers. The procedure of immune-staining was carried out according to the protocols of Dako

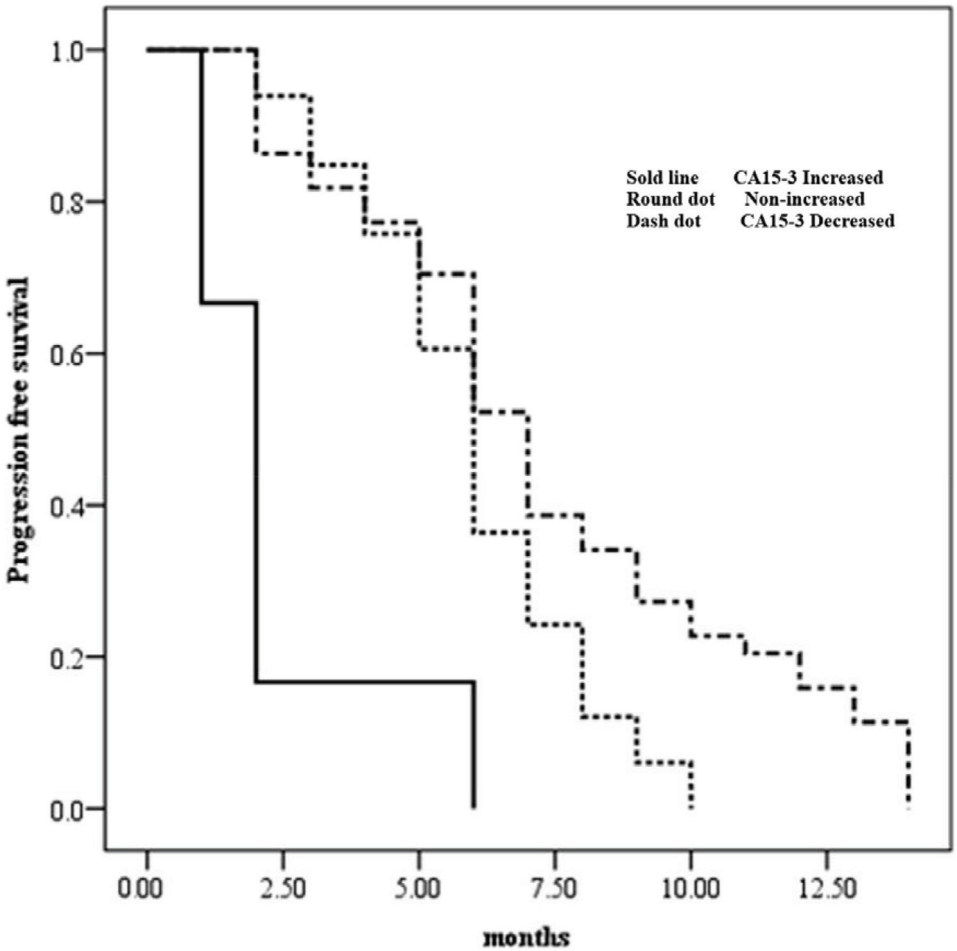

Figure 3. Kaplan-Meier graph showing progression-free survival with CA15-3 changes after two cycles of systemic therapy in the patients with metastatic breast cancer ($P < 0.001$).

cytomation (manufacturerís protocols). Briefly, the procedure consisted of using alcohol and xylene for deparafinization followed by rehydration of the sections by antigen retrieval solution (95ºC for 40 minutes). Afterwards, we applied 3% peroxide for 5 minutes. Followed by applying primary antibodies (ER, PR, c-erbB-2, and Ki-67) of Dako type, incubation was performed for 30 minutes. Subsequently, HRD solution was added for 30 minutes and the staining process was completed by incubation with DAB (diaminobenzidine) for 10 minutes. Finally, we performed staining of the background with hematoxyline stain. With each immunohistochemical run, appropriate external and internal controls were added. The slides were examined by two qualified histopathologists.

Examination and assessment of the ER and PR slides were done by selecting 100 tumor cells. The positive cell ratio to the total cell was determined and recorded as percentages. Furthermore, the staining intensity was reported. Depending on the ASCO and CAP instructions, the tumors with staining of 1% or more of invasive cancer cells, were regarded as positive. The report also included the average intensity of the stain (weak, moderate, or strong).[15,16,17]

Regarding HER2 staining, the scoring of the staining patterns of cases were as follow: 0, 1+, 2+, and 3+ (ASCO/CAP guidelines). HER2 0/1+

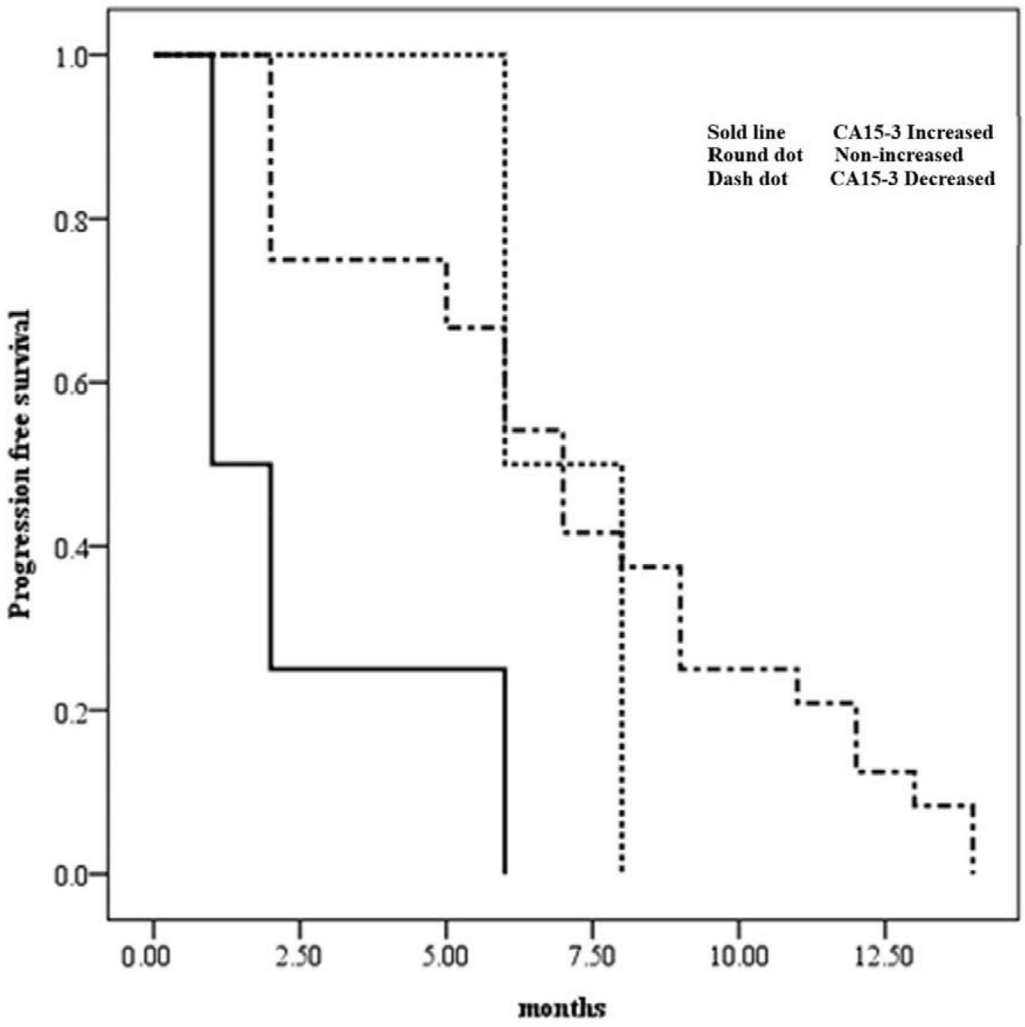

Figure 4. Kaplan-Meier graph showing progression-free survival with CA15-3 changes after two cycles of systemic therapy in the patients with Luminal subtypes of metastatic breast cancer ($P = 0.007$).

score was reported to be negative and HER2 3+ score was positive, while those cases with 2+ score were reported to be equivocal, which were further subjected to FISH in order to confirm the HER2 status.[18]

Regarding Ki67, the examination of an average of 500 cancer cells' nuclei was done, and the results were expressed as the percentage of positive cells.[15, 19]

Molecular classification was carried out for all the patients based on immunohistochemical expression of ER, PR, HER2, and Ki67. Accordingly, the patients were categorized as follow: Luminal A (ER+, PR+, HER2-, Ki67 ≤ 20); Luminal B (ER+, PR+, HER2-, Ki67 > 20); Luminal B/HER2+(ER+, PR+, HER2 +); HER2 enriched (ER-, PR-, HER2+); triple negative breast cancer (ER-, PR-, HER2-).[15]

Measurement of tumor marker CA15-3

For CA15-3 tumor marker, serum CA15-3 level was determined using an automated immunoassay system (MINI-VIDAS, biomerieux company, French). We carried out the procedure according to the manufacturer's instructions. The cut-off value of CA15-3 level was the increment (or decrement) of 25 U/ml.

Follow-up

All the patients were followed up regularly at an interval of three weeks for at least 16 months and were treated by endocrine therapy,

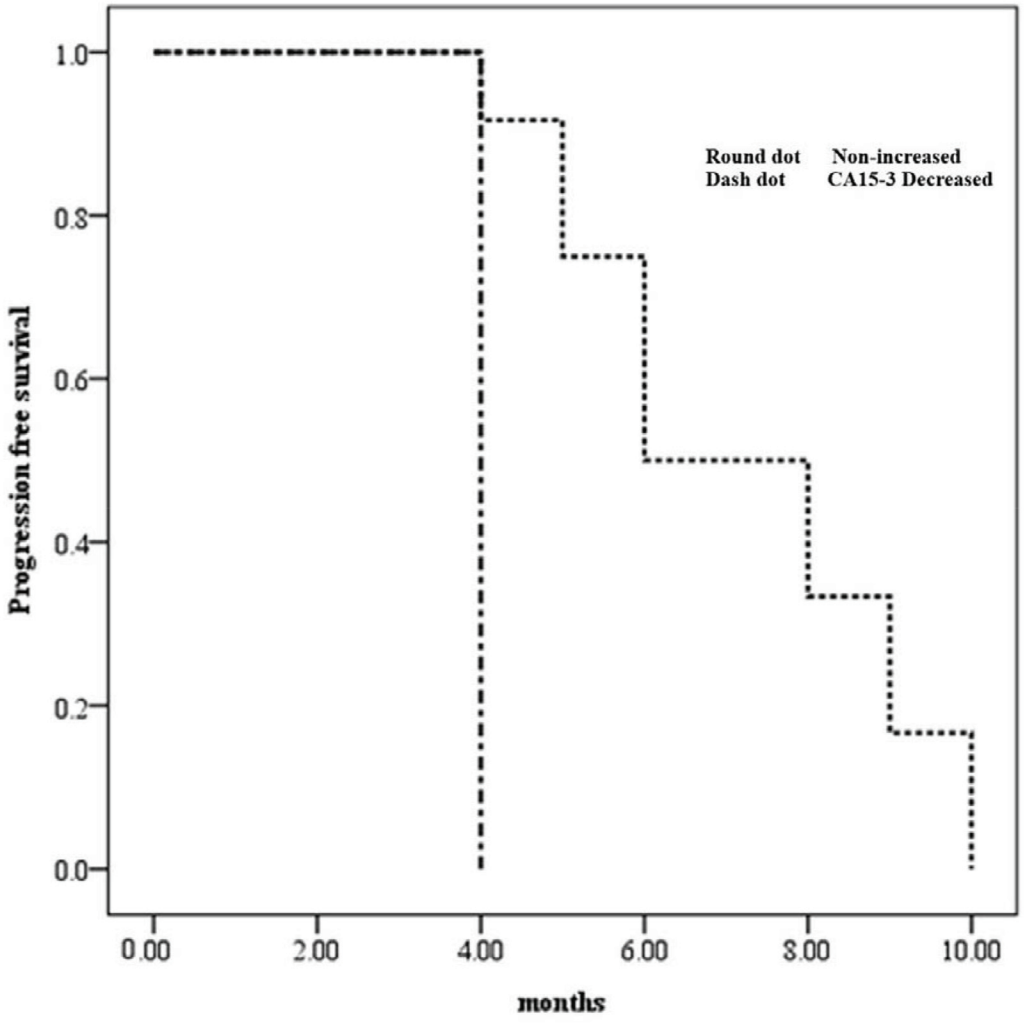

Figure 5. Kaplan-Meier graph showing progression-free survival with CA15-3 changes after two cycles of systemic therapy in the patients with triple negative subtype of metastatic breast cancer ($P = 0.005$).

Table 1. The correlation between age and metastatic sites and CA15-3 levels in 83 subjects with metastatic breast cancer

	No.	Mean CA15-3 level	SE	SD	*P* value
Age (years)					
≤35	15	112.4	32.71	126	0.41
≥35	68	100	10.86	86	
Metastasis No.					<0.00
Single	69	84	8.9	74	
Multiple	14	193.7	36.9	138	
Metastatic site					
Brain					0.035
Yes	15	55.06	13.47	52.19	
No	68	112.9	12.2	101.9	
Lung					0.009
Yes	43	129.02	17.6	115.5	
No	40	74.03	9.5	60.3	
Liver					0.014
Yes	40	129.31	17.4	110.5	
No	43	77.6	11.3	74.4	

No.: Number; SD: Standard deviation; SE: Standard error

chemotherapy, target therapy, and/or bisphosphonate according to the international guidelines.[20] CA15-3 levels were measured before starting the systemic therapy and evaluated every 3 weeks during the course of therapy.

Response evaluation to the therapy in those patients were assessed after two cycles of systemic therapy by clinical and two medical radiological tests based on RECIST criteria.[21] The response was classified into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Progression-free survival (PFS) was calculated based on the time of diagnosis of metastatic breast cancer to the time of disease progression in months.

To investigate the relationship between the levels of CA15-3 response to systemic therapy and PFS, the patients were divided into two groups based on their CA15-3 levels prior to starting the systemic therapy (normal and elevated). Subsequently, the patients were allocated to three groups based on the relative changes in the levels of CA15-3 at the end of the second cycle of the systemic therapy: the increased group (an increment of CA15-3 by 10% or higher relative to pretreatment levels), the non-increased group (an increment of CA15-3 level by less than 10% from pretreatment level or a reduction of CA15-3 level by less than 10% relative to pretreatment level), and the decreased group (a reduction of CA15-3 level by 10% or higher relative to pretreatment level).

Statistical analysis

We performed the statistical analysis and data management via SPSS (Version 20; SPSS). For the assessment of the significant differences in the levels of CA15-3 in the patients with different molecular subtypes, χ^2 test was used. Kaplan-Meier method was employed for verifying the relationship between CA15-3 and PFS.

Results

The distribution of different molecular subtypes of breast cancer, in our study, was as follows: 13 cases of Luminal A (15.7%), 32 cases of Luminal B (38.6%), 16 cases of Luminal B/HER2+ (19.3%), 8 cases of HER2 enriched (9.6%), and 14 cases of triple negative (16.9%).

The correlations between CA15-3 levels and different molecular subtypes in the 83 cases of breast cancer were analyzed. Figure 1 represents this analysis, in which Luminal subtypes are shown to be more frequently associated with elevated CA15-3 at the time of diagnosis of metastasis. CA15-3 is elevated in (91%) of Luminal cases compared with HER2 enriched (50%) or triple negative cases (21%). Furthermore,

Table 2. CA15-3 changes after two cycles of systemic therapy in the metastatic breast cancer patients with different molecular subtypes

Molecular	Increased	Non-increased	Decreased	Total	*P* value
Luminal A	0	3	10	13	$P < 0.001$
Luminal B	4	4	24	32	
Luminal B + HER2+	2	6	8	16	
HER2 enriched	0	8	0	8	
Triple negative	0	12	2	14	
Total	6	33	44	83	

HER2: Human epidermal growth factor receptor 2

large magnification of CA153 could be observed in Luminal subtypes compared with HER2 enriched and triple negative subtypes with $P < 0.001$.

Out of the 83 subjects, 69 (83.13%) had single organ metastasis, while 14 (16.87%) had multiple organ metastasis, as shown in table 1. The patients with multiple organ metastasis had significantly higher CA15-3 levels compared with those with single organ metastasis with $P < 0.001$.

The serum levels of CA15-3 were elevated in the patients with lung or liver metastases relative to those without lung or liver metastases with $P = 0.009$ and $P = 0.014$, respectively. On the contrary, no correlation was observed between the level of CA15-3 and brain metastasis, as depicted in table 1.

To assess the predictive value of CA15-3 levels in monitoring the response to systemic therapy in different molecular subtypes of metastatic breast cancer, the changes in the levels of CA15-3 were evaluated subsequent to the second cycles of systemic therapy and categorized as increased, non-increased, and decreased groups.

We found that the patients with Luminal subtypes showed a significant reduction in the levels of CA15-3 after systemic therapy compared with non-Luminal subtypes with $P < 0.001$, as demonstrated in table 2.

According to figure 2, no significant association was found between the level of CA15-3 at the time of diagnosis of metastasis and PFS. However, the mode of the changes in the levels of CA15-3 after two cycles of systemic therapy was observed to be a predictive tool for prognosis. We found that the patients with an increased levels of CA15-3 after these two cycles has a significantly shorter PFS than those with non-increased or decreased levels of CA15-3 (2.33 months versus 5.95 months or 7.38 months). Figure 3 illustrates that these changes were observed not only in Luminal subtypes of breast cancer (Figure 4), but also in the triple negative subtype (Figure 5). Moreover, we found that the decrease in the levels of CA15-3 was significantly correlated with the achievement of good clinical response after the systemic therapy; whereas, the increase in the levels of CA15-3 was significantly associated with poor response, as shown in table 3.

Discussion

Herein, we evaluated the mode of the changes in CA15-3 levels following the systemic therapy in different molecular subtypes of breast cancer. We aimed to determine CA15-3 reliability in predicting prognosis and treatment monitoring in comparison with both clinical and radiological evaluations of cancer, which have been used routinely for the assessment of cancer burden in metastatic breast cancer. The most widely used criteria is the RECIST (Response Evaluation Criteria for Solid Tumor) that was adapted by academic and cooperative institutions in 2000.[21] Although this criterion depends on the information obtained from medical imaging, diffuse micrometastasis is sometimes difficult to visualize through imaging;[9,21] therefore, we focused on finding a tumor marker which could be more predictive for progression of the disease process than imaging tests.

CA15-3 remains the most frequently utilized circulating tumor marker in breast cancer and its clinical value is attributed to CA15-3 association with tumor burden, like tumor size and lymph node status.[22,23] Moreover, Lee et al. observed that CA15-3 elevation was more frequent in

Table 3. Correlation between CA15-3 changes and clinical response after two cycles of systemic therapy in metastatic breast cancer

Group	PR	SD	PD	Total	*P* value
CA15-3 Increased	0	0	6	6	$P < 0.001$
CA15-3 Non increased	13	17	3	33	
CA15-3 Decreased	24	12	8	44	
Total	37	29	17	83	

PR: Partial response; SD: Stable disease; PD: Progressive disease

metastatic breast cancer patients than in early breast cancer. Additionally, patients with an elevated CA15-3 levels before tumor resection showed a more frequent elevation of CA15-3 at recurrence.[24] Similarly, Colomer et al. demonstrated that cases with multiple metastatic lesions had a higher level of CA15-3 than those with single metastatic lesion,[25] which is consistent with our results.

The benefit of CA15-3 level measurement in predicting prognosis and response to therapy in breast cancer remains controversial. ESO-ESMO guidelines recommend the observation of CA15-3 levels for monitoring response evaluation in metastatic breast cancer.[26] Similarly, NAGB and EGTM panels recommend the measurement of CA15-3 before each chemotherapy cycle and at three months interval in patients receiving endocrine therapy for response evaluation in cases with metastatic breast cancer.[27,28] Nevertheless, ASCO panel recommends that CA15-3 measurement be used only for monitoring the clinical parameters in metastatic breast cancer.[29] These controversies may be due to conflicting conclusions of various investigations.[30,31] Kim HS et al. evaluated the clinical implication of CA15-3 slope of regression exponential curves derived from serial CA15-3 measurements and interestingly concluded that CA15-3 levels kinetics was a good predictive prognostic index, especially in terms of recurrence.[32] Similarly, Laessig et al. reported that the regular measurement of CA15-3 levels could provide useful information about early detection of recurrence.[33]

There are conflicting results regarding the correlation between metastatic site and CA15-3 level. Berruti et al. showed that the prevalence of elevated CA15-3 levels varied with metastatic sites and patients with visceral metastasis had a more frequent elevation of CA15-3 than the others with soft tissue and bone metastasis.[34] Meanwhile, He Zhen ya et al. indicated that patients with elevated CA15-3 levels were more prone to bone and abdominal metastasis.[35] Other studies could not find any significant differences concerning CA15-3 levels between different metastatic sites.[36] In the present research, since we only included the patients with metastatic lesions measurable with RECIST (excluding those patients with bone metastasis, Ascites, pleural /pericardial effusion), we found that CA15-3 elevation was more frequent in patients with liver and lung metastasis compared with that in other metastatic sites. Similar results were reported by Yang yue et al. who demonstrated that CA15-3 elevation was strongly associated with liver metastasis.[37] Further researches are required to investigate the relation between CA15-3 levels and metastatic sites, which may be of great value for predicting prognosis.

It is well known that molecular classification of breast cancer based on ER, PR, HER2, and Ki67 is widely used for determining a therapeutic approach to the treatment of metastatic breast cancer and that it is regarded as a remarkable prognostic factor.[14] However, the association between serum CA15-3 level and molecular subtyping of breast cancer is not well known although a strong correlation has been reported between ER positivity and CA15-3 level in metastatic breast cancer.[11] Nonetheless, other studies did not find any statistical differences concerning CA15-3 levels in patients with different molecular subtypes of early stages and locally advanced breast cancer.[38]

Our study demonstrated that CA15-3 levels were associated with different molecular subtypes of metastatic breast cancer at the time of the initial diagnosis of metastasis. In addition, CA15-3 elevation was found to be more frequently observed in Luminal subtypes than in HER2

enriched and triple negative subtypes, which is in line with the results of other studies.[39] This could be also fully related to the nature of mature luminal cells presented in Luminal subtypes, which were specified by a high expression of ER and PR genes,[40] whereas less differentiated subtypes of breast cancer (HER2 enriched and triple negative) lack certain circulating genes.[40]

Duffy MJ. et al. reported that CA15-3 level was an independent prognostic factor for the overall survival in breast cancer patients with different stages.[41,42] However, our study found no relation between CA15-3 level and PFS in metastatic breast cancer at the time of diagnosis; this finding is consistent with those reported by others.[43] The aforementioned result is quite reasonable since CA15-3 is more often elevated with Luminal subtypes of breast cancer, which have a better general prognosis compared with other subtypes.[14] Hence, the prognostic factor of CA15-3 at the time of diagnosis of metastasis can only be considered for Luminal subtypes rather than for all breast cancer patients.

Furthermore, in our prospective study, we found that reduction in CA15-3 level by 10%, after two cycles of systemic therapy (chemotherapy, endocrine therapy, or target therapy) was correlated to longer PFS and achievement of good clinical response in different molecular subtypes of breast cancer. On the other hand, increment in the level of CA15-3 predicted shorter PFS and indicated poor clinical response to the therapy.

Conclusion

The mode of the change in CA15-3 levels is closely correlated with the clinical therapeutic response and survival advantage rather than the pretreatment level of CA15-3 in metastatic breast cancer especially in Luminal subtypes. Therefore, the measurement of CA15-3 level at regular intervals after starting the systemic therapy showed the same quality as medical imaging at lower cost. This measurement could predict clinical response and replace imaging examinations, like CT scan, PET, and MRI, which have been used routinely for monitoring response in patients with Luminal subtypes of metastatic breast cancer.

Acknowledgment

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Conflict of Interest

None declared.

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