

Original article:

Role of Pre-Operative Serum CA 15-3 Level in risk stratification of Breast Cancer

* Kaustubh S Chaudhari¹, Prashant S Patil¹, Sumeet S Pawar², Nikhil N Tambe³

¹Department of Medical Oncology, IPGME&R and SSKM Hospital, Kolkata, India.

² Lokmanya Tilak Municipal Medical College, Mumbai, India.

³ M.G.M. Medical College, Navi Mumbai, India.

Corresponding author:

Dr. Kaustubh S. Chaudhari ; EMail ID-kaustubhschaudhari@gmail.com

Abstract:

Background: Cancer Antigen 15-3, a derivative of a large transmembrane glycoprotein polymorphic epithelial mucin derived from MUC1 gene is a reliable marker in identifying patients who would benefit most from adjuvant therapy.

Materials & Methods: Aimed at assessing the overall risk, possibility of distant metastasis and need for adjuvant therapy we assessed the role of pre-operative CA 15-3 and compared it with Nottingham's Prognostic Index (NPI) and the established prognostic markers like tumor size, lymph node status, histological grade, development of distant metastasis, death from disease.

Results: We found no significant association between elevated pre-op CA 15-3 tumor size, histological grade and lymph node status when assessed separately, however, when these prognostic factors were combined together as *Nottingham Prognostic Index* (NPI), a significant association was established and also associated with a low 5 year survival rate. Patients with high pre-op CA 15-3 were the ones who developed distant metastasis in follow-up of 2 years and died due to breast cancer.

Conclusions: Patients with elevated CA 15-3 preoperatively had poor outcome reflected by high NPI and early recurrence and this group of patients is most likely to benefit from *adjuvant therapy*. Patients in high risk group have significant risk of developing distant metastasis; low 5 year survival rate, high risk of death from disease and require intensive surveillance for early detection of distant metastasis.

Keywords: Cancer Antigen 15-3 (CA 15-3), Nottingham Prognostic Index (NPI), Nottingham Prognostic Index (NPI)

INTRODUCTION

Breast cancer is the second most common cancer in Indian women constituting 18 % of total cancer burden. It is the most common cancer in urban women with incidence of 22-28 per 1,00,000 women per year. The incidence of breast cancer is rapidly rising, particularly in metro cities. While managing Indian patients of breast cancer we need to consider financial condition of patients and limited availability of Government hospitals providing adjuvant therapies. Hence it is important to predict patients most likely to have poor outcome and benefit most from adjuvant therapy. If we have a prognostic marker which can reliably predict patients with poor outcome, high risk of recurrence,

we can use adjuvant therapy judiciously and rationally. Traditional tumor markers in breast cancer like tumor size, lymph node status, histological grade need tumor sampling, are costly, depend on competence and expertise of cyto / histopathologist. On the other hand, serum is easily accessible; measuring marker is simple, objective, reproducible, and cost-effective. Soluble circulating marker, if found to be accurate prognostic marker, would be ideal candidate for predicting outcome and monitoring treatment course¹. The potential role of CA 15-3 in prognosis has been studied in a few investigations which came to inconsistent conclusions². In the present prospective study, we analyzed

the relation between pre-op serum CA 15-3 level and patient outcome.

MATERIALS AND METHODS:

Patient selection: All female primary breast cancer patients treated at Breast Clinic, Dept. of General Surgery, IPGME&R and SSKM Hospital, Kolkata from 2011 to 2012 had their pre-op serum CA 15-3 measured and it was repeated on 7th, 30th post-op day and every 6 monthly for 2 years. Patients were excluded if any other malignancy was known from their previous history or if staging investigations at the time of diagnosis revealed evidence of distant metastasis. A total of 55 patients fulfilled these criteria. 20 female patients with benign breast diseases were taken as age matched controls and serum CA 15-3 measured for comparison.

Treatment: Patients were treated with either modified radical mastectomy (MRM) or quadrantectomy and axillary lymph node dissection with local radiotherapy (RT). After completion of surgery, RT and appropriate adjuvant chemotherapy or hormone therapy was not altered according to marker levels but was administered as indicated based on international guidelines.

Marker Analysis: CA15-3 serum levels were determined by IMMULITE 2000 BR-MA (Medical System), a two steps sequential chemoluminescent immunometric assay that used an anti-CA15-3 mouse monoclonal antibody. The lower limit of sensitivity was 0.2 U/ml and the established cut-off was 25 U/ml. According to cut-off, patients were divided in two subgroups: a) subgroup one consisting of 3/55 patients with CA 15-3 serum levels <25 U/ml and b) subgroup two consisting of 52/55 patients with CA 15-3 >25 U/ml.

Follow of Patients: Clinical follow-up included history taking, physical examination and lab tests including serum CA 15-3, liver function tests, complete blood count, chest radiography, abdominal and breast USG, mammography for detection of local or distant relapse. Additional computed tomography and radiography were obtained as necessary.

STATISTICAL ANALYSIS

Categorical variables were expressed as number of patients and percentage of patients and compared across the 2 groups using Freeman-Halton extension of Fisher's exact test. Continuous variables are expressed as Mean \pm Standard Deviation and compared across the 2 groups using unpaired t test. An alpha level of 5% was taken, i.e. a p value less than 0.05 was considered significant.

RESULTS

Patients with benign breast diseases showed average serum CA 15-3 level of 10.7 U/ml.

Table No. 1 shows serum CA 15-3 level in pre-op and post-op period in study group. 52 of the 55 patients in study group had raised pre-op serum CA 15-3. Out of 55, 43 patients showed decline in serum CA 15-3 post-operatively. 6 patients had persistent raised serum CA 15-3 post-operatively. However pre-op serum CA 15-3 values were much higher than post-op values in these 6 patients.

Table 2 shows that, 38 of the 55 patients with histological grade 2 or 3 had raised pre-op CA 15-3. Only 1 patient out of 21 with grade 2, had normal serum CA 15-3. None of the grade 3 patients had normal serum CA 15-3. 41 of the 55 patients with tumor > 2 cm in size had raised pre-op CA 15-3. None of the patients with tumor size more than 5 cm had normal pre-op serum CA 15-3; while only 1 out of 19 patients with tumor 2-4.99 cm in size had normal serum CA 15-3. 41 of the 55 patients with metastasis in 4 or more axillary lymph nodes had elevated pre-op CA 15-3. None of the patients with metastasis in > 9 axillary lymph nodes had normal serum CA 15-3.

Pre-op serum CA 15-3 was elevated in patients with large tumor size, higher number of axillary lymph nodes with metastasis and higher histologic grade. This may indicate total tumor burden shown by raised serum CA 15-3 level.

However, Test of proportion showed that pre-op serum CA 15-3 had no significant association with tumor size, nodal status or histological grade (p > 0.05).

NPI is an indirect prognostic indicator. We analyzed relation between pre-op serum CA 15-3 and NPI.

[$NPI = (0.2 \times \text{tumor size}) + (1 \text{ for histologic grade } 1, 2 \text{ for grade } 2, 3 \text{ for grade } 3) + (1 \text{ for no axillary node metastasis}, 2 \text{ for } 1-3 \text{ metastatic nodes}, 3 \text{ for } >3 \text{ metastatic axillary lymph nodes})$].

Table 3 shows; 41 of the 52 patients with raised pre-op serum CA 15-3 had $NPI > 4$. Three patients with normal pre-op CA 15-3 had $NPI < 4$. While none of patients with higher NPI had normal pre-op serum CA 15-3.

Test of proportion showed significant association between raised pre-op serum CA 15-3 and high NPI.

As shown in Table No.4; Five of these patients developed distant metastasis and one patient died due to breast cancer. The result was significant for distant metastasis but not for death from cancer.

DISCUSSION

Traditional prognostic markers such as axillary lymph node status, tumor size, histological grade, hormone receptor expression are helpful to stage the disease, predict overall survival of patient and response to hormonal therapy. Multigene analysis³ and gene expression profiling⁴ have been studied recently to know more about biological behavior of breast cancer. However, all these factors need tissue sampling, are costly and results depend on expertise of histopathologist. None of these factors can single handedly predict risk of development of distant metastasis in individual patient, overall survival of patient and patients needing close surveillance and follow-up. We also need a marker which can identify the group of patients benefitting most from adjuvant therapy.

Soluble circulating tumor marker if found to be accurate prognostic factors, would be ideal candidates for predicting outcome and monitoring treatment response¹. Serum is easily accessible, result does not depend on individual expertise and the test is economical. Serum CA 15-3 has been the most frequently investigated tumor marker in breast cancer. Due to low sensitivity and specificity, CA 15-

3 has no value for primary diagnosis⁵⁻⁸. It can, however be useful in predicting prognosis, measuring treatment response in advanced breast cancer patients⁹⁻¹² and for early detection of metastasis¹³⁻¹⁶.

As discussed in results, we found that patients with elevated serum CA 15-3 in pre-op period and showed persistent raised serum CA 15-3 after R⁰ resection were at increased risk of distant metastasis and risk was significant. Possibly high serum CA 15-3 levels were due to release of tumor associated antigen from occult metastasis in systemic circulation indicator of vascularisation of tumor and bad prognosis from beginning.

By comparing pre and post operative values, we found decline in post-op values in majority of patients (43/55). The post-op serum CA 15-3 values were comparable to median of healthy individuals which could be expected as investigated only patients with complete resection of tumor (R⁰ resection). We found significant association between raised pre-op CA 15-3 and higher NPI score. 5/41 patients with raised pre-op CA 15-3 and $NPI > 4$ developed distant metastasis and 1 patient from this group died due to breast cancer, while none of patient with low pre-op serum CA 15-3 and $NPI < 4$ developed distant metastasis or died from breast cancer. So CA 15-3 can risk stratify patients in high risk and low risk group. Patients in high risk group have significant risk of distant metastasis and statistically non-significant risk of death from disease. Patients in high risk group need close follow-up and most likely benefit from adjuvant therapy.

Velaiutham S et al¹⁷ in their retrospective study of 437 women who had CA15-3 levels determined at initial presentation of breast cancer found that of those patients who were adequately staged, CA15-3 was found to be elevated (defined as $> 51 \text{ U/ml}$) in 0% of Stage 1, 7.9% of Stage 2, 36.7% of Stage 3 and 68.6% of Stage 4 cases. In a subset of 331 patients with survival data, patients with normal CA15-3 had an 85% five year overall survival rate compared to 38% in their counterparts with elevation of the

tumor marker. The level of elevation was also significantly related to survival; patients with values more than 200 U/ml exhibited only a 28% five year survival. The association of elevated CA15-3 at initial presentation with poor outcome was maintained over univariate and multivariate analyses. Our results correlated with them as estimation of CA15-3 at presentation of breast cancer is important as it is an independent prognostic indicator and may prompt the physician to investigate for metastases if elevated.

Despite some controversies, CA 15-3 level could provide independent prognostic information to be taken together with conventional markers measured in tumor tissues¹⁶. Furthermore, Duffy¹⁸ reported that pre-op concentration could be combined with existing early treatment based exclusively on increasing marker concentration showed improved prognosis compared with controls¹⁹⁻²³. However we do not recommend early treatment based solely on raised serum CA 15-3 and suggests randomized controlled trial for

role of adjuvant therapy in patients with raised tumor marker.

CONCLUSION

This study showed that elevated pre-op serum CA 15-3 was significantly associated with high NPI and thus can predict poor patient outcome. Patients with raised serum CA 15-3 after R⁰ resection were at significant risk of recurrence, CA 15-3 can risk stratify breast cancer patients in high risk and low risk group.

To our knowledge, this is the first study in Indian patients reporting on high prognostic relevance of tumor associated antigen CA 15-3 in primary breast cancer patients. An advantage of our approach for clinical would be the independence from tumor tissues. In addition to those prognostic factors being already indicative for adjuvant therapy like lymph nodes, histological grade, a randomized prospective therapy intervention study based on decrease of CA 15-3 would be needed to prove the relevance of our finding.

Table 1. Showing pre-op and post-op serum CA 15-3 values in breast cancer patients.

| Serum CA 15-3 | Pre-op value | 7 th post-op day | 30 th post-op day | After every 6 months for 2 years |
|---------------|--------------|-----------------------------|------------------------------|----------------------------------|
| < 25 U/ml | 3 | 43 | 48 | 43 |
| >25 U/ml | 52 | 12 | 7 | 6 |

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Table No.2 Showing relation between pre-op CA 15-3 and tumor size number of axillary lymph nodes positive for metastasis, number of patients with different histological grades and no. of patients with NPI scores.

| | | CA 15-3 level | | | | Comparison using χ^2 (P value) |
|------------------------------|-----------|---------------|------|----------|------|-------------------------------------|
| | | <25 U/ml | % | >25 U/ml | % | |
| Grade | I | 2 | 12.5 | 14 | 87.5 | 0.374 |
| | II | 1 | 4.7 | 20 | 95.2 | |
| | III | 0 | 0 | 18 | 100 | |
| Tumor size (cm) | <2 | 2 | 14.3 | 12 | 85.7 | 0.229 |
| | 2 - 4.99 | 1 | 5.3 | 18 | 94.7 | |
| | >5 | - | 0 | 22 | 100 | |
| Lymph node metastasis | 1-3 nodes | 2 | 14.3 | 12 | 85.7 | 0.175 |
| | 4-9 nodes | 1 | 3.8 | 25 | 96.2 | |
| | >9 nodes | - | 0 | 15 | 100 | |
| NPI | 2-4 | 3 | 21.5 | 11 | 78.5 | 0.027* |
| | 5-7 | - | 0 | 27 | 100 | |
| | > 7 | - | 0 | 14 | 100 | |

*- statistically significant

Table 3. Shows relation between pre-op serum CA 15-3 and different NPI.

| Pre-op serum CA 15-3 vs. NPI | 2-4 | 5-7 | >7 |
|------------------------------|-----|-----|-----|
| <25U/ml | 3 | --- | --- |
| >25U/ml | 11 | 27 | 14 |

Table 4. Shows relation between NPI score and number of patients developing distant metastasis and number of patients dying from breast cancer.

| | No. of patients | No. of patients who developed distant metastasis | No. of patients who died due to breast cancer |
|-----------------------------------|-----------------|--|---|
| Low risk group (NPI<4) | 14 | 0 | 0 |
| High risk group (NPI>4) | 41 | 5 | 1 |

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