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CYCLOOXYGENASE-2 EXPRESSION IN POST-MASTECTOMY CHEST WALL RELAPSE.

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The purpose of this study was to assess the prognostic significance and clinical correlations of cyclooxgenase-2 expression (COX) in a cohort of patients treated with radiation (RT) for post-mastectomy chest wall relapse (PMCWR). Between 1975 and 1999, 113 patients were treated for isolated PMCWR. All patients were treated with biopsy and/or excision of the CWR followed by RT. Median follow-up was 10 years. All clinical data including demographics, pathology, staging, receptor status, HER-2/neu status, and adjuvant therapy were entered into a computerized database. Paraffin-embedded CWR specimens were retrieved from 42 patients, of which 38 were evaluated, created into a tissue microarray, stained by immunohistochemical methods for COX, and graded 0-3+. A score of 2-3+ was considered positive. Overall survival from original diagnosis for the entire cohort was 44% at 10 years. Survival rate after chest wall recurrence was 28% at 10 years. The distant metastasis-free survival rate after CWR was 40% at 10 years. Local-regional control of disease was achieved in 79% at 10 years after CWR. COX was considered positive in 13 of 38 cases. COX was inversely correlated with ER ($p = .045$) and PR ($p = .028$), and positively correlated with HER-2/neu ($p = .003$). COX was also associated with a shorter time to PMCWR. The distant metastasis-free rate for COX negative patients was 70% at 10 years, compared with 31% at 10 years for COX-2 positive patients ($p = 0.029$). COX positive had a poorer local-regional progression-free rate of 19% at 10 years, compared with 81% at 10 years for COX negative ($p = 0.003$). Outcome following RT for PMCWR is relatively poor. Positive COX correlated with other markers of poor outcome including a shorter time to local relapse, negative ER/PR and positive Her-2/neu status. Positive COX correlated with higher distant metastasis and lower local-regional control of disease. If confirmed with larger studies, these data have implications with respect to the concurrent use of COX-2 inhibitors and radiation for PMCWR.

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INTRODUCTION

Breast cancer is the leading cancer among women and the second most common cause of cancer death in women after lung cancer. It is the main cause of death in women ages 45 to 55. [(1)] Breast cancer rates have increased by 1.2 % per year from 1940 to 1980. Each year, approximately 180,000 American women are diagnosed with breast cancer and 40,000 die from the disease. [(1)] Although the most important risk factor for the development of breast cancer is age, risk may be affected by age at menarche, first pregnancy, age at menopause, use of exogenous estrogens, and family history. A variety of pathologic findings may be discovered at the time of diagnosis, but the most important distinction from the standpoint of treatment has been between invasive and non-invasive (in situ) cancer. Invasive (infiltrating) ductal carcinoma is the most common type of breast cancer, while ductal carcinoma in situ (DCIS) comprises approximately 15-20% of all breast cancer. Although early detection and improved treatment modalities over the years have increased survival rates, extensive efforts have been directed at improving outcomes with more targeted therapies. Various factors may affect the treatment of localized breast cancer. These include the size and extent of the tumor, spread of cancer to lymph nodes, tumor markers, and hormone receptors. Current treatment options for breast cancer are often integrated into a multi-modality approach by including various combinations of surgery, chemotherapy, hormone therapy, and radiation therapy. Regarding chemotherapy, anthracycline-based regimens are often chosen due to their significant benefit compared to nonanthracycline-containing adjuvant chemotherapy such as cyclophosphamide, methotrexate, plus fluorouracil, or CMF.[(1)] Surgery may involve

mastectomy or lumpectomy (breast-conserving surgery) with or without lymph node dissection. Lumpectomy is usually accompanied by radiation therapy to the entire breast. Despite the increasing use of breast conserving surgeries for breast cancer, mastectomy still plays a large role in primary and salvage treatment.

Unfortunately, despite the attempted removal of all breast tissue during a mastectomy, local-regional recurrences (LRR) still occur. About 10-20% of all patients develop a local recurrence, mostly within the boundaries of the chest wall, within a 10 year period despite achieving negative surgical margins. [2-9] Boundaries of the chest wall extend from the supraclavicular line to the diaphragm, and from the sternum to the mid-axillary line. Local-regional recurrence may occur in the skin, subcutaneous tissue, muscle, or bone of chest wall. The three most common sites of local-regional recurrence in decreasing order are the chest wall, supraclavicular region, and axilla. The most frequent site for recurrence in the chest wall is in the scar or grafted area from previous mastectomy. Most local recurrences occur within a 3 year period after mastectomy. [10]

Local-regional recurrences are significant because they often cause considerable morbidity and distress to patients. A recurrence may present as a rash-like lesion, induration, nodule or ulcer. Poor circulation and relatively rapid tumor growth may prolong healing time for lesions such as ulcers. In one study by Bedwinek et al, 62% of patients with uncontrolled LRR experienced one or more of the following problems: bleeding and/or ulceration, pain, arm edema, and brachial plexus paralysis.[11]

Another study by Bedwinek et al attempted to identify the natural history after LRR and to identify clinical factors that would predict which subsets of patients would have a good prognosis.[12] A retrospective study was performed of 129 patients with chest wall relapse after mastectomy who then received radiation only, excision only, or both. Factors that determined good prognosis were single recurrence, size of largest recurrence less than 1 cm, and a disease-free interval of greater than 2 years. All of these factors had a significant effect on disease-free survival and overall survival. Overall survival and disease-free survival at 5 years from primary diagnosis were found to be 36% and 13% respectively, while 81% of patients eventually developed distant metastases. The time to develop distant metastases was longer for patients with good prognostic factors.

A large study performed by Recht et al in 1999 demonstrated the correlation between the number of positive nodes and tumor size at the time of primary diagnosis to incidence of local-regional failure. [13] Among patients with 1-3 positive lymph nodes at primary diagnosis, the 10-year cumulative incidence of isolated local-regional failure increased with increasing tumor size. Among patients with T2 stage, the 10-year cumulative incidence of local-regional failure more than doubled between those who had 1 to 3 positive lymph nodes compared to those who had 4 to 7 positive lymph nodes. These results show that the incidence of local-regional failure was positively correlated with number of positive lymph nodes and tumor size.

Treatment for chest wall relapses often consists of surgical excision when feasible followed by radiation therapy to the area of recurrence. Patients are treated up

to 50 Gy for completely excised recurrent tumors. For unresected lesions, 60-65 Gy is given for lesions less than 3 cm while 65-75 Gy is given for lesions greater than 3 cm.

Prognosis is poor for patients who develop a chest wall relapse. Five-year postrecurrence survival rates range from 36% to 53% [4, 5, 9, 12, 14-19] while disease-free survival rates range from 13% to 44%.[4, 12, 16, 18, 19] Thus, these women represent a high-risk subgroup with respect to systemic recurrence and mortality. In addition to this, these patients also face significant morbidity as stated before due to the aggressive nature of their tumors. Although various clinical and pathological parameters have been indicated as prognostic factors for disease-free and overall survival after local-regional recurrence, there is a need to examine molecular markers as prognostic tools to aid clinical management.

One molecular marker that has been the subject of active research is cyclooxygenase-2 (COX-2). COX-2 has been identified as a tumor marker associated with poor prognosis in various cancers based on epidemiological, preclinical and translational studies. Cyclooxygenase is a well-known enzyme in the inflammatory pathway that catalyzes the conversion of arachidonic acid to prostaglandin H_2, the rate-limiting step in the formation of prostaglandins from membrane phospholipids. There are two forms of cyclooxygenase: COX-1 and COX-2. COX-1 is known to be constituitively expressed in a variety of mammalian cells. Its role has been linked to physiological functions such as the cytoprotection of the stomach and control of platelet aggregation.[20] In contrast, COX-2 is thought to be upregulated in response to growth factors, tumor promoters, cytokines, and several oncogenes.[21-23]

Active research has been directed at exploring the association between COX-2 and carcinoma. As a result, COX-2 has been found to be overexpressed in various cancers such as lung, cervical, head and neck, colon, and pancreas. [24-29] One study evaluated the prognostic significance of elevated COX-2 expression in a cohort of primary resected lung adenocarcinomas.[24] A significant relationship was established between increased COX-2 expression and shortened patient survival only in a cohort of patients with stage I disease ($p = 0.034$). In one study by Gaffney et al, increased expression of COX-2 was found to be associated with poorer overall and disease-free survival in a cohort of cervical carcinoma patients treated with definitive radiation therapy.[28] Another study evaluating squamous cell carcinoma of head and neck cancers (HNSCC) demonstrated that mean levels of COX-2 mRNA were increased by nearly 150-fold in HNSCC compared with normal oral mucosa from healthy volunteers.[30] A study evaluating pancreatic cancers demonstrated increased expression of COX-2 protein in pancreatic carcinomas compared to benign tumors. [29] Knocking out the COX-2 gene in murine models of familial adenomatous polyposis was shown in a study by Oshima et al to decrease the number and size of intestinal polyps.[31] Expression of COX-2 has also been studied in human transitional cell carcinoma of the urinary bladder.[32] COX-2 immunoreactivity was detected in 66% of the carcinomas, whereas only 25% of the non-neoplastic samples were positive ($p < 0.005$).

Furthermore, there has been increasing evidence of the role of COX-2 in breast cancer. COX-2 is expressed in about 40% of human breast cancers, and is associated with poor prognosis.[33] The possible role of COX-2 in breast cancer was

first noted when elevated levels of prostaglandins were found in breast tumor cells.[34-36] A study in rat models subsequently demonstrated that forced overexpression of COX-2 induced breast cancer.[37] Translational studies have also confirmed the positive correlation between these two parameters. Ristimaki et al found that 37.4% of invasive cancers analyzed by immunohistochemistry were found to have positive COX-2 expression. In another study, 43% of invasive cancers and 63% of ductal carcinomas in situ (DCIS) were found to stain positively for COX-2 by immunohistochemical methods.[38] Positive COX-2 expression has also been correlated with decreased distant disease-free survival, large tumor size, high proliferation rate, and human epidermal growth factor receptor 2 (HER-2) gene amplification.[20] These studies suggest that COX-2 inhibition may play a potential role in breast cancer treatment.

STATEMENT OF PURPOSE

The main objective of this analysis was to retrospectively determine whether a significant correlation exists between positive COX-2 expression at the time of chest wall recurrence and prognosis for post-mastectomy breast cancer patients using tissue microarray analysis. Prognosis was determined by percentage of overall survival, distant metastasis-free survival, and local-regional progression-free survival. Secondary objectives were to examine the relationship between COX-2 expression and time to post-mastectomy chest wall relapse, and ER, PR, and HER-2/neu status at the time of local-regional recurrence.

PATIENTS and METHODS

A retrospective review was conducted of 113 patients who were treated with radiation therapy for chest wall recurrence at the Yale University Department of Therapeutic Radiology between 1979 and 1999. All patients had an isolated chest wall recurrence without evidence of distant metastases at the time of presentation. Patients who presented with primarily lymph node metastases, patients with simultaneous distant metastases, and patients who received prior radiation therapy were excluded from the analysis.

Patients underwent surgical excision of the local-regional recurrence when feasible, followed by external beam radiation therapy. Standard radiation treatments were given, and patients were treated to a total median dose of 60 Gray (Gy) to the chest wall. Nineteen patients received radiation treatment to the chest wall only, and the remaining patients received radiation to the chest wall and regional lymphatics. Adjuvant chemotherapy and hormone therapy were administered as deemed necessary by physicians. Forty patients received chemotherapy, and 56 patients received hormonal therapy. The median follow-up after treatment for recurrence was 10.13 years.

Patient charts were reviewed for demographics and radiation therapy parameters. Other data including clinical and pathologic staging of initial tumor, method of detection, lymph node status, histologic parameters, surgery performed, adjuvant treatment for the initial tumor, estrogen receptor status, progesterone receptor status, chest wall progression, distant metastases, disease-free survival, and overall survival were documented. All data were entered into a computerized database. The

protocol for chart review and processing of tumor specimens was approved by the Yale School of Medicine Human Investigations Committee.

Tumor blocks from chest wall recurrence specimens were successfully collected from 42 patients, of which 38 were evaluable for tissue microarray analysis. The tissue microarray was created by taking core needle sections from existing tumor blocks and arranging them into a common paraffin block. This recipient paraffin block was prepared with 2-fold redundancy, which denotes acquiring two samples from each of the pre-existing paraffin-embedded tumor specimens. Two-fold redundancy has been shown to correlate well with conventional immunohistochemical staining. One study determined that analysis of two cores was comparable to analysis of a whole tissue section in greater than 95% of cases.[39] It has also been demonstrated that many proteins in archival tissue retain their antigenicity for greater than 60 years, thus validating the use of these tissues in microarray analysis.

Cores were initially made in the recipient block to allow placement of tumor specimens. Core depths were made at approximately 2-3 mm while diameters were set at 0.6 mm. "Biopsies" were then taken from the donor block and then placed into the cores of the recipient block. These were performed until specimens from all 38 patients were placed into the recipient paraffin block. The block was then incubated at 37 degrees for 10 minutes to allow the cores to adhere to the walls of the holes in the array. The array block was then placed into a microtome for sectioning and cut 5 uM thick with a tape-based tissue transfer system (Intrumedics, Hackensack, NJ, U.S.A.) After each section was placed on Paraffin Sectioning Aid (PSA-4X) adhesive coated

slides, they were radiated with UV light for 20 seconds. The slide was then placed in xylene for 60 seconds and allowed to air dry.

Analysis by immunohistochemistry was performed on microarray slides after deparaffinization in xylene followed by 100% ethanol. Samples were then pretreated to promote antigen retrieval with the DAKO Target Retrieval Solution (DAKO, Carpinteria, CA, U.S.A.) A 3% hydrogen peroxide solution was then used for endogenous peroxidase blocking. Slides were then incubated with monoclonal antibody COX-2 (Cayman Chemical, Ann Arbor, MI, U.S.A.; #160112; dilution 1:50) After incubation, the slides were washed in phosphate buffered saline, and a biotinylated secondary antibody was applied. Samples were then applied with DAKO streptavidin-horseradish peroxidase using LSAB + Kit. DAKO DAB (3,3-diaminobenzidine tetrahydrochloride dehydrate) was then applied as a chromogenic substrate. Tissue microarrays were also stained for HER-2/neu, ER, and PR from a previous study that used patients from a similar cohort as this study to create a microarray.[40]

Analysis of the tissue staining was performed by a single pathologist (V.B.) and one of the authors who were both blinded to patient outcome. Both distribution (percentage of tumors stained) and intensity of the cytoplasmic staining were documented. Intensity was recorded on a scale of 0 to 3+ with 3+ having the strongest intensity. The specimens were then given a positive or negative score based on the intensity and distribution scores. Any score with 2 or 3+ with a distribution of >10% was considered positive in this study, in accordance with standard clinical practices.[41]

These data were added to the computerized database with all clinical and pathologic outcomes variables.

Chart review for data collection, collection of chest wall recurrence tissue for tissue microarray analysis, and reading of slides with pathologist for scoring were performed by the student. Creation of tissue microarray and staining of prepared slides by Cox-2 antibody were performed by the tissue microarray lab and immunohistochemistry lab respectively at Yale University School of Medicine.

The Prodas Data Base System (Conceptual Software, Houston, TX) was used to assess patient data and statistics. The Cox regression model was used to test clinical and pathologic factors by both univariate and multivariate analysis. The life-table method was used to calculate survival curves and the Mantel-Hanszel chi-square test to measure differences in survival curves. This generation of data was performed by the thesis advisor.

RESULTS

One hundred thirteen patients presented to the Department of Therapeutic Radiology at Yale-New Haven Hospital between January 1979 and December 1999 for radiation treatment to their chest wall for local-regional recurrence of breast cancer. The mean +/- standard deviation (SD) age at initial diagnosis for these 113 patients was 52.3 years +/- 13.7 years. The mean +/- SD age at presentation with a first isolated chest wall recurrence was 56.9 years +/- 14.5 years. The mean time to the initial chest wall recurrence was 4.6 years. The median follow-up after initial diagnosis was 13.7 years, and the follow-up after treatment for local-regional recurrence was 10.1 years.

Of 113 patients, 98 were Caucasian, 10 were African American and 5 were from other racial groups. All patients received simple, modified, or radical mastectomy for their initial tumor. The mean +/- SD size of the tumor at initial diagnosis of breast cancer was 3.3 cm +/- 2.1 cm. The mean number of axillary lymph nodes sampled was 14.4 +/- 9.2 lymph nodes, with a mean +/- SD of 3.7 +/- 6.9 positive lymph nodes. Half of the patients (n = 57) received adjuvant chemotherapy and 33 patients received adjuvant hormone therapy at the time of diagnosis of their original breast carcinoma. The mean +/- SD time between initial diagnosis and chest wall recurrence was 4.6 years +/- 4.6 years. Within 5 years, 66% of patients had experienced their recurrence; and, by 10 years, 90% of patients had developed a recurrence. At the time of chest wall relapse, the mean +/- SD size of the recurrence tumor was 1.6 cm +/- 1.0 cm (median, 1.5 cm; range, 0.5-5.0 cm; n = 78). Clinical outcomes for entire patient population are summarized in Table 1. Overall survival

after original diagnosis was 69% at 5 years and 44% at 10 years. Overall survival after chest wall recurrence was 46% at 5 years and 28% at 10 years. The distant metastasis-free rate for all patients after chest wall recurrence was 49% at 5 years and 40% at 10 years. Local progression-free survival after chest wall recurrence was 83% at 5 years and 79% at 10 years.

Table 2 summarizes the demographic, staging, receptor status, and treatment parameters at both initial diagnosis and time of chest wall relapse for the cohort of 38 patients included in the tissue microarray. Mean age at diagnosis was 56.7 years with a range from 35-92 years. Pathologic T status at the time of primary diagnosis was found to be predominantly T2 with the second most common status being T3. Patients were also predominantly found to have negative lymph node status at the time of original diagnosis (23/38). There was one patient with unknown lymph node status. Estrogen and progesterone receptor status were approximately equally distributed among positive, negative, and unknown status. The majority of patients received modified radical mastectomy at the time of primary diagnosis (34/38) as opposed to total mastectomy (3/38) or radical mastectomy (1/38). At the time of chest wall relapse, patients predominantly underwent excisional biopsy (33/38). Most patients also did not receive chemotherapy at the time of recurrence (30/38), although most received hormone therapy (25/38). There were no significant differences between this selected cohort of 38 patients and the overall group of 113 patients with respect to the major clinical characteristics and outcomes. The 5-year overall survival after primary diagnosis of all patients (n=113) was 69% while those in the array (n=38) was 78%. The 5-year distant metastases-free survival after primary diagnosis was 69% of all

patients compared to 67% of those in the array. Finally, the 5-year local progression-free survival after chest wall relapse was 83% for all patients compared to 73% of those in the array.

Cox-2 staining was predominantly cytoplasmic. Using the criteria of 2-3+ staining in greater than 10% of cells, 13 of 38 (34%) were considered positive. The majority of patients underwent complete excision of their recurrence tumors (33/38). Those who underwent biopsy were in complete remission following external beam radiation therapy. Only 8/38 patients received adjuvant chemotherapy while 25/38 patients were known to receive hormone therapy at the time of chest wall relapse. There were no significant differences in treatment (surgery, chemotherapy or hormonal therapy) as a function of COX-2 status (Table 3). Among patients with positive COX-2 status, 12 of 13 (92%) received an excisional biopsy at the time of chest wall relapse while among those with negative COX-2 status, 21 of 25 (84%) underwent this procedure. Nine of 13 (69%) of positive COX-2 patients received hormone therapy at the time of chest wall relapse while 16 of 22 (73%) of negative COX-2 patients received this therapy. Finally, 3 of 13 (23%) of positive COX-2 patients received chemotherapy at chest wall relapse while 5 of 25 (20%) of negative COX-2 patients underwent this route.

Correlation of COX-2 expression with various molecular markers, disease-free interval, and chest wall progression is summarized in Table 4. COX-2 expression was found to be positively correlated with HER-2/neu status, early time to chest wall recurrence, and chest wall progression. All were statistically significant. When HER-2/neu status was analyzed, 9 of 14 patients (64%) with positive HER-2/neu status had

positive COX-2 expression. This compares with only 5 of 14 patients (36%) with positive HER-2/neu status who had negative COX-2 expression. In regards to time to chest wall recurrence, patients were divided into two groups of early recurrence and late recurrence. Early recurrence was defined as <24 months. Seven of 11 patients (64%) who tested positive for COX-2 demonstrated early recurrence while only 6 of 27 patients (22%) who tested positive for COX-2 showed late recurrence. In chest wall progression analysis, 7 of 11 (64%) of patients who tested positive for COX-2 expression developed another chest wall recurrence after treatment for their original chest wall relapse, while 6 of 27 (22%) of positive COX-2 patients did not.

COX-2 expression was negatively correlated with both ER and PR status, both with statistical significance. When ER status was analyzed, 5 of 23 (22%) of patients with positive ER status demonstrated positive COX-2 expression. This was in contrast to 18 of 23 (78%) of patients with positive ER status demonstrating negative COX-2 expression. In regards to PR status, only 2 of 15 (13%) of patients with positive PR status demonstrated positive COX-2 expression while 13 of 15 (87%) demonstrated negative COX-2 expression.

Evaluations of COX-2 expression in relation to outcomes are summarized in Table 5. It is worth noting that the 5-year distant metastases-free rate after primary diagnosis for COX-2 negative patients was 79% while the rate for COX-2 positive patients was 41% (Figure 1). This was significant with a p-value of 0.029. The five year local progression-free rate for COX-2 negative patients was 87% while the rate for COX-2 positive patients was 38% (Figure 2). This was highly significant with a p-value of 0.003.

A multivariate model, taking into account all molecular markers (Her-2/neu, ER, and PR), as well as time to recurrence was performed on the 38 patients. COX-2 overexpression was still found to be a significant predictor of chest wall progression (HR = 4.91; CI = 1.09-22.1; P = 0.038). In the analysis for distant metastases free survival, significant predictors were COX-2 expression (HR = 5.25; CI = 1.44-19.2; P = 0.01) and positive HER-2/neu status (HR = 0.22; CI = .054-0.92; P = 0.038). PR status demonstrated borderline significance (HR = 0.29; CI = 0.075-1.12; P = 0.073).

DISCUSSION

Despite receiving treatment for local-regional recurrence after mastectomy, prognosis is poor for these patients and much research has been directed at determining the factors that lead to this poor prognosis. The location, extent and size of recurrence, number of recurrences, number of recurrence nodules, primary lymph node status, ER status of primary, ER and PR status of recurrence, and age at time of recurrence have all been reported to be indicators of prognosis.[4, 5, 9, 12, 17-19] A large number of studies have reported that the most significant factor for distant metastases and survival is the disease-free interval, or interval to recurrence from primary diagnosis. Decreased disease-free interval, or early recurrence has been associated with ultimately poor outcome in the majority of these studies.[4, 5, 9, 12, 17-19, 42, 43] Despite these clinical and pathological prognostic parameters, there is a need to determine molecular markers to more accurately guide clinical management. Unfortunately, it has been difficult to accrue an adequate number of patients for large prospective randomized studies following women after chest wall recurrence. As a result, Haffty et al conducted a retrospective study in order to explore the prognostic significance of estrogen receptor, progesterone receptor, p53, HER-2/neu, and cyclin D at chest wall relapse. Significant factors for distant metastasis after local recurrence were time to recurrence and PR status, and for local-regional disease progression was HER-2/neu status.[40] The distant metastasis-free survival rate for patients with positive PR status was 84%, compared to 38% in those with negative PR status. Patients with positive HER-2/neu status had a local-regional progression-free rate of 59% while patients with negative HER-2/neu status had a rate of 92%. Additional

biological markers need to be assessed to more clearly define prognosis and assist the physician in selecting therapy regimens catered for high-risk patients. One marker that has been the focus of active investigation is COX-2.

In our study, a uniform cohort of patients who developed chest wall relapse and received radiation therapy were selected to assess the prognostic significance of COX-2 after relapse. Although there was no significant difference in overall survival between COX-2 positive and negative patients, patient numbers were relatively small to detect survival differences. The resulting analysis demonstrated that patients who overexpressed COX-2 had a lower chance of being free of distant metastases and higher chance of chest wall progression both on univariate and multivariate analysis. Patients with positive COX-2 expression had a 5-year distant metastases-free rate of 41% while those with negative COX-2 expression had a rate of 79% after primary diagnosis. The results of the 5-year local progression free rate was even more significant at 38% with positive COX-2 expression and 87% with negative expression. These poor prognostic findings are consistent with a previous study that found the distribution and intensity of COX-2 expression from tissue microarray analysis to correlate significantly with diminished disease-free survival in breast cancer patients, although in this study COX-2 also correlated with decreased overall survival.[(44)] Paraffin-embedded tumor specimens from 23 women with invasive cancer were stained for COX-2 expression. The 5-year overall survival rate was 100% for patients with less than 75% of breast cancer cells expressing COX-2 protein compared with 49% for patients with greater than or equal to 75% expression ($p = 0.044$). The study also found that younger patients with invasive breast cancer may have a greater

percentage of COX-2 expression in their cancers. A significant association has also been found between COX-2 overexpression and distant metastasis in breast cancer.[45] In this study, archival specimens of human breast cancer (n = 29) were collected and analyzed after staining with COX-2 antibodies. COX-2 overexpression occurred in 37.9% of their sample study. Previous studies have also demonstrated that positive COX-2 expression is likely associated with poor outcome due to its correlation with poor prognostic factors such as large tumor size, high histological grade, axillary node metastases,[20] and lymphovascular invasion.[46] Furthermore in our study, COX-2 positivity more likely resulted in early local-regional recurrence, or time to recurrence in less than 2 years (P = 0.015). This is significant because as stated before, early recurrence has been associated with poor prognosis. COX-2 was also found to be negatively correlated with ER (P = 0.045) and PR status (P = 0.028) at recurrence in our analysis. This finding is consistent with two recent studies that also analyzed COX-2 expression using tissue microarray analysis in breast cancer.[20, 46] Because positive ER[47] and PR status[48] has been found to be associated with good prognosis, this inverse association with these two markers most likely indicates poor prognosis for this cohort of patients.

A marker that occurs in 20-30% of human breast cancers and has been correlated with poor prognosis is HER-2/neu.[49-53] Press et al demonstrated that HER-2/neu gene amplification in the absence of adjuvant therapy is an independent predictor of poor clinical outcome and is a stronger predictor than tumor size. Patients with small tumors who demonstrated HER-2/neu gene amplification were at increased risk of recurrence and disease-related death.[51] Another study by Seshadri et al

concluded that HER-2/neu amplification is an independent predictor of shorter disease-free survival in both node-negative and node-positive patients.[52] Our results demonstrated that COX-2 expression was positively correlated with HER-2/neu status ($P = 0.003$), thereby indicating poor prognosis. Other studies have also confirmed a positive correlation between these two markers. Two recent studies showed that elevated COX-2 expression was correlated with the presence of HER-2 oncogene amplification in human breast cancers.[20, 54] Howe et al demonstrated that treatment with celecoxib, a COX-2 inhibitor, significantly reduced the incidence of mammary tumors in mice overexpressing wild-type neu protein and caused about a 50% reduction in mammary prostaglandin E2 (PGE2) levels. Because mammary glands from these mice models expressed all four PGE2 receptor subtypes, it was suggested that signaling through PGE2 receptor subtypes is important for the development of mammary tumors.[55] Benoit et al further showed that COX-2 inhibition reduced HER-2/neu proteins levels and acted synergistically with trastuzumab, an anti-HER-2 monoclonal antibody in breast cancer cell lines.[56] Concurrent administration of COX-2 inhibitors and HER2/neu antibodies for treatment experimental colorectal cancer inhibited tumor growth more effectively than when either was administered alone.[57]

More pertinent to our study is the possibility that COX-2 expression may be involved with decreased radiosensitivity during treatment of recurrence. Other studies have shown this relationship between COX-2 and radiosensitivity. In vitro studies demonstrated that a selective COX-2 inhibitor, SC-236, in glioma tumor cell culture medium enhanced cell killing by ionizing radiation. When administered in

combination with local radiation, SC-236 caused a greater than additive increase in tumor growth delay.[58] Two recent reports concluded that SC-236 markedly increased tumor radioresponse in NFSA[59] and FSA sarcoma[60] bearing mice without greatly affecting normal tissue radioresponse. Other studies have shown that prostaglandins may serve as radioprotectors.[61] Although the mechanism is not well understood, it is thought that prostaglandins may protect cellular repair mechanisms[62] or inhibit radiation-induced apoptosis. Additional studies should be performed to elucidate the association of COX-2 expression and diminished radiosensitivity.

The methods in our study included use of tissue microarray, which was composed of 0.6 mm cores from original tissue specimens. The validity of using these cores to represent larger tissues has been confirmed in a variety of cancers such as head and neck, lung, and breast.[39, 63, 64] An advantage to using this technique was that all specimens were processed simultaneously using the same conditions during immunohistochemistry, thus resulting in experimental uniformity. Tissue specimens collected at chest wall relapse were relatively homogeneous. Chest wall specimens were only collected from patients who did not present with simultaneous distant metastases. A monoclonal COX-2 antibody was used which may have improved specificity for COX-2 compared with a polyclonal antibody.

There were also some limitations to this study. Although the method of immunohistochemistry is relatively fast and readily available in most pathology departments, it is a test subject to interobserver variability. The reading of stained microarray slides is also subjective and may result in variability among pathologists.

Other limits of this study included its retrospective design and relatively small sample size. Additional studies need to be carried out to validate these findings.

Due to the poor prognosis associated with increased COX-2 expression in tumors, COX-2 inhibitors as potential therapeutic targets remain an active area of research. Various selective COX-2 inhibitors have been shown to slow tumor growth in experimental animals.[65, 66] Kishi et al reported that SC-236 was found to inhibit tumor growth on its own, decrease PGE2 levels in sarcoma FSA tumors, and inhibit neoangiogenesis. One study showed that NS-398, a COX-2 selective inhibitor, induced apoptosis in colorectal tumor cells which was independent of COX-2 protein expression.[67] Celecoxib has been shown to decrease tumor size when compared to control groups in rat models.[68] In another study by Masferrer et al, COX-2 was found to suppress tumor growth by inhibiting angiogenesis. COX-2 was detected in the angiogenic vasculature in most of human colon, prostate, lung, and breast tumors. In addition, celecoxib dose-dependently inhibited tumor growth and the number and size of lung metastases in two animal models of Lewis lung carcinoma and the human colon carcinoma HT-29. It is noteworthy that the expression of COX-2 in these models was mainly limited to the angiogenic blood vessels, the preexisting vasculature adjacent to the primary tumor, and the blood vessels invading the metastatic lesions, and not the cancer cells themselves. In the same study, celecoxib was also a potent inhibitor of angiogenesis in the rat corneal model.

Numerous mechanisms have been proposed attempting to elucidate the correlation between COX-2 expression and tumorigenesis. One theory suggests that COX-2 catalyzed products such as prostaglandins may directly break down into a

mutagen and form adducts with deoxynucleosides.[69] Prostaglandins, more specifically PGE_2, are also known to be potent immunosuppressants. PGE2 blocks the anti-tumor activity of macrophages and natural killer cells,[70, 71] and inhibits production of cytotoxic lymphokines. These activities may block natural surveillance mechanisms to inhibit tumor growth and spread. COX-2 has also been hypothesized to induce carcinogenesis through regulation of apoptosis. Rat intestinal epithelial cells transfected with COX-2 showed increased adhesion to extracellular matrix, resistance to butyrate-induced apoptosis, and elevated expression of bcl-2, a protein which inhibits apoptosis.[72] COX-2 may also affect tumorigenesis by dysregulating cell growth. It has been proposed that increased duration of G1 phase of the cell cycle may cause resistance of cells that permanently express COX-2 to undergo programmed death.[73] It has also been suggested that COX-2 is correlated with angiogenesis. COX-2 showed a significant linear correlation ($P = 0.001$) with staining of CD31, an endothelial cell marker of angiogenesis in breast cancer tissue.[74] CD31, which is also known as platelet-endothelial cell adhesion molecule-1, is thought to play a role in various endothelial cell functions including angiogenesis, migration, and transmigration of leukocytes across endothelium. This correlation with angiogenesis is significant because some studies have suggested that angiogenesis may be inversely associated with survival.[75]

In conclusion, our study showed that for patients who develop a local-regional recurrence after mastectomy, positive COX-2 expression at the time of recurrence is a sign of poor prognosis. These patients have a lower chance of expressing favorable prognostic markers such as ER and PR, and a higher chance of expressing poor

prognostic markers such as HER-2/neu. In addition, they have a greater possibility of local progression and distant metastases. These results may suggest that COX-2 may play a role in decreased radiosensitivity at the time of local-regional relapse, and that COX-2 inhibition with or without anti-HER2/neu therapy during chest wall radiation may improve prognosis. More specifically, COX-2 inhibitors may provide a relatively safe and inexpensive adjuvant to chest wall irradiation. Prospective randomized trials with a large cohort of patients are needed to further assess these results.

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Table 1
Outcomes and Survival Data for 113 Patients

Endpoint	Actuarial rate at 5 yrs +/- SE (%)	Actuarial rate at 10 yrs +/- SE (%)
Overall survival after chest wall recurrence	46 +/- 4.9	28 +/- 4.8
Distant metastasis-free survival after chest wall recurrence	49 +/- 5.3	40 +/- 5.9
Local progression-free survival after chest wall recurrence	83 +/- 3.6	79 +/- 4.1

SE: standard error

Table 2

Clinical Characteristics of 38 Patients in Tissue Microarray

Characteristic	No. of patients	Mean	Range
Age at diagnosis (yrs)	38	56.7	35-92
Follow-up from original diagnosis (yrs)	38	21.1	7-45
T status at original diagnosis			
Pathologic T status			
T1	7		
T2	17		
T3	8		
T4	1		
Unclear original clinical T status	5		
Lymph node status at original Dx			
No. with positive lymph nodes	14		
No. with negative lymph nodes	23		
Unknown	1		
Receptor status at original diagnosis			
Estrogen receptor			
Positive	13		
Negative	14		
Unknown	11		
Progesterone receptor			
Positive	12		
Negative	12		
Unknown	14		
Surgery performed			
Total mastectomy	3		
Modified radical mastectomy	34		
Radical mastectomy	1		
Adjuvant systemic therapy			
Hormone therapy			
Given	11		
None given	27		
Chemotherapy			
Given	12		
None given	26		
Surgery at CWR			

Excisional biopsy	33
Incisional biopsy	5
Chemotherapy at CWR	
Given	8
None given	30
Hormone therapy at CWR	
Given	25
None given	10
Unknown	3

Table 3

Correlation of Cox-2 with Clinical Characteristics of 38 Patients

Characteristic	COX-2 Pos	COX-2 Neg
Surgery at CWR		
Excisional biopsy	12/13	21/25
Incisional biopsy	1/13	4/25
Hormone Therapy at CWR		
Given	9/13	16/22
Not given	4/13	6/22
Chemotherapy at CWR		
Given	3/13	5/25
Not given	10/13	20/25

CWR: chest wall relapse

Table 4

Correlation of COX-2 with ER, PR, HER-2/neu, Time to CWR, and Chest wall progression

	COX-2: Pos	COX-2: Neg	P-value
ER Status			
Pos	5/23 (22%)	18/23 (78%)	0.045
Neg	8/15 (53%)	7/15 (47%)	
PR Status			
Pos	2/15 (13%)	13/15 (87%)	0.028
Neg	11/23 (48%)	12/23 (52%)	
HER-2/neu			
Pos	9/14 (64%)	5/14 (36%)	0.003
Neg	4/24 (17%)	20/24 (83%)	
Time to CW Recurrence			
Early (< 2 yrs)	7/11 (64%)	4/11 (36%)	0.015
Late (> 2 yrs)	6/27 (23%)	21/27 (77%)	
CW progression			
Pos	7/11 (64%)	4/11 (36%)	0.015
Neg	6/27 (22%)	21/27 (78%)	

ER = estrogen receptor; PR = progesterone receptor; Pos = positive; Neg = negative; CW = chest wall

Table 5
Correlation of COX-2 with Outcomes

Endpoint	COX-2 Pos	COX-2 Neg	P-value
Five yr survival after CWR (%)	47	56	.32
Five yr distant mets-free rate after primary dx (%)	41	79	.029
Five yr local progression-free rate after CWR (%)	38	87	.003

Pos: positive; Neg: negative; yr: year; dx: diagnosis; CWR: chest wall relapse; mets: metastases

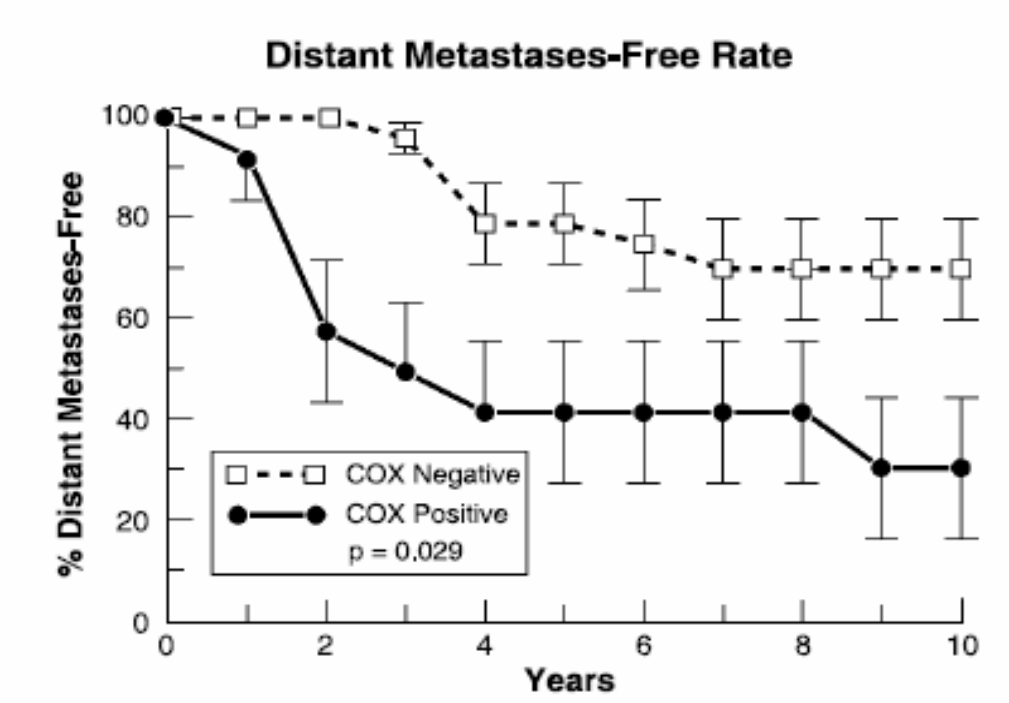

Figure 1. Distant metastasis free rate following chest wall relapse by COX-2.

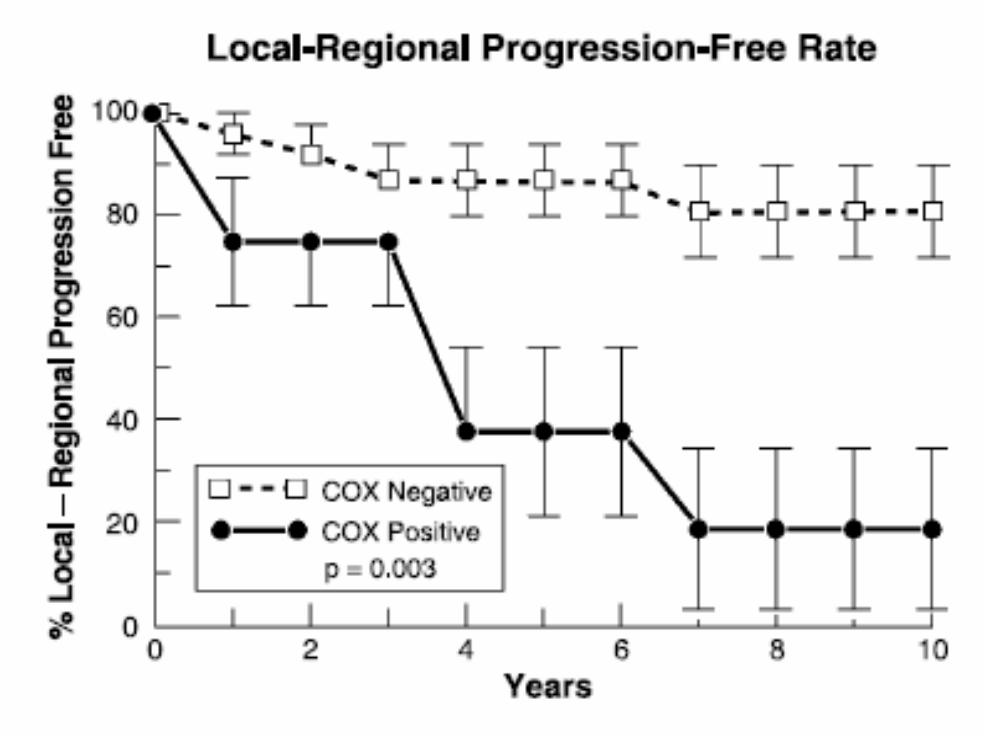

Figure 2. Local chest wall progression free rate by COX-2.