Pathological responses and long-term outcome analysis after neoadjuvant chemotherapy in breast cancer patients from Kuwait over a period of 15 years

Yamini Krishnan,a Shafika A. Alawadhi,b Sreedharan P.S.,a Murali Gopal,a Sanjay Thuruthela

From the aMedical Oncology, Kuwait Cancer Control Centre, Kuwait, Kuwait; bFaculty of Medicine, Kuwait University, Kuwait, Kuwait

Correspondence: Dr. Yamini Krishnan · Medical Oncology, Kuwait, Kuwait · T: +96524847608 · yams23in@yahoo.com

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BACKGROUND AND OBJECTIVES: The attainment of pathological complete response (pCR) after neoadjuvant chemotherapy has been taken as a surrogate marker for disease-free survival and overall survival. This is however dependent on various other parameters such as stage, grade, and biologic markers.

DESIGN AND SETTINGS: This is a retrospective study of 365 patients with histologically confirmed non-metastatic breast cancer patients treated with neoadjuvant chemotherapy at the Kuwait Cancer Control Centre between 1998 and 2009.

PATIENTS AND METHODS: A total of 365 breast cancer patients who had received neoadjuvant chemotherapy from 1998–2009 were analyzed for the relationship of pCR with hormone status, Her2 status, histopathological subtype. Survival analysis was also conducted.

RESULTS: Hormone receptor (HR) negative tumors had a higher pCR as against HR positive tumors, and the highest pCR in our analysis of pathological subtypes were seen in the HR+, Her2neu + and HR−, Her2neu + group. In our study, we could make out the paradoxes that well differentiated, and HR positive tumors had a better survival in spite of having lower pCR. The luminal A subtype also had a better overall survival than the triple negative subtype in spite of having lower pCR with neoadjuvant chemotherapy.

CONCLUSION: Though the achievement of pCR retains its significance, it is more prognostic in HR negative tumors. The importance of HR receptor status, grade, and histopathological subtype in the long-term survival has been emphasized.
The patients had all undergone core biopsy for diagnosis along with ER, PR, and Her2 testing. They were staged according to American Joint Committee on Cancer Guidelines (AJCC), and the medical records were reviewed for clinical parameters and survival assessment.

Histopathological examination
All the tumors were classified into different subtypes by the WHO classification. The histopathological records were reviewed for ER, PR, and Her2neu results. ER and PR statuses were assessed by immunohistochemistry (IHC) studies with a threshold of 10% or more to be classified as positive (which was the standard during the period of our study). Tumors with either ER or PR positivity were taken as hormone receptor (HR) positive. Her2 was assessed by IHC according to the Herceptin scoring system and a score 3+ was taken as positive. Patients with an equivocal IHC score of 2+ had fluorescence in situ hybridization (FISH) amplification done with a threshold Her2neu/CEP17 ratio ≥2 taken as positive or amplified. As a protocol all histopathological and IHC slides were independently reviewed by 2 pathologists.

Treatment
Anthracycline- and taxane-containing combinations were employed in the neoadjuvant schedules. Most patients received either anthracycline-containing (42/365-11.5%) or anthracycline- and taxane-containing regimens (302/365-82.7%) with a few patients receiving taxane only (16/365-4.3%) or non-anthracycline non-taxane regimens (5/365-1.3%). The chemotherapy regimens used were either FEC (fluorouracil 500 mg/m² IV, epirubicin 100 mg/m² IV, and cyclophosphamide 500 mg/m² IV on day 1, every 3 weeks), AC (doxorubicin 60 mg/m² IV and cyclophosphamide 600 mg/m² IV on day 1, every 3 weeks), TE (docetaxol 75 mg/m² with epirubicin 75 mg/m² every 3 weeks), FEC-D (4 cycles of FEC followed by 4 cycles of docetaxol 80-100 mg/m² day 1, every 3 weeks), AC-T (4 cycles of AC followed by weekly taxol 80 mg/m² for 12 weeks or 175 mg/m² every 3 weeks), or TE (docetaxol 75 mg/m² with epirubicin 75 mg/m² every 3 weeks) for 6 to 8 cycles. Among the total 103 patients with Her2 positive disease, maintenance trastuzumab was given to 72 patients but only 44 among them received neoadjuvant trastuzumab. Trastuzumab was either given as 4 mg/kg initial loading dose followed by 2 mg/kg maintenance weekly or 8 mg/kg loading dose followed by 6 mg/kg maintenance 3 weekly for a total period of 1 year. All patients under-

Table 1. Patient characteristics and pCR.

|                       | Total No (%) | pCR | pCR (%) | P value |
|-----------------------|--------------|-----|---------|---------|
| Overall               | 365          | 50  | 13.7    |         |
| Age of patient        |              |     |         |         |
| <50 years             | 183 (50.2%)  | 24  | 13.1%   | .745    |
| >50 years             | 182 (49.8%)  | 26  | 14.2%   |         |
| Menstrual status      |              |     |         |         |
| Premenopausal         | 237 (64.9%)  | 36  | 15.1%   | .26     |
| Postmenopausal        | 128 (35.1%)  | 14  | 10.9%   |         |
| Clinical stage of disease |         |     |         |         |
| IIA/IIB               | 41 (11.2%)   | 6   | 14.6%   | .90     |
| IIIA/IIB/IIC          | 305 (83.5%)  | 40  | 13.1%   |         |
| Inflammatory          | 19 (5.3%)    | 4   | 21%     |         |
| Clinical lymph node   |              |     |         |         |
| Present               | 324 (88.7%)  | 45  | 13.8%   | .766    |
| Not present           | 41 (11.3%)   | 5   | 12.1%   |         |
| Histopathological type|              |     |         |         |
| IDC                   | 338 (92.6%)  | 48  | 14.2%   | 0.542   |
| ILC                   | 19 (5.2%)    | 1   | 5.2%    |         |
| Others                | 8 (2.2%)     | 1   | 12.5%   |         |
| Grade of tumor        |              |     |         |         |
| Grade I               | 29 (7.9%)    | 0   | 0%      | .009    |
| Grade II              | 141 (38.6%)  | 13  | 9.2%    |         |
| Grade III             | 149 (40.8%)  | 28  | 18.7%   |         |
| Unknown               | 46 (12.7%)   | 9   | 19.5%   |         |
| Hormone receptor (HR) status |       |     |         |         |
| HR+                   | 214 (58.6%)  | 22  | 10.2%   | .024    |
| HR–                   | 151 (41.4%)  | 28  | 18.5%   |         |
| Her 2 status          |              |     |         |         |
| Her2+                 | 103 (28.2%)  | 22  | 21.3%   | .027    |
| With trastuzumab      | 44           | 15  | 34%     |         |
| Without trastuzumab   | 59           | 7   | 11.8%   |         |
| Her2–                 | 239 (65.5%)  | 26  | 10.8%   |         |
| Her2 unknown          | 23 (6.3%)    | 2   | 8.6%    |         |
| Pathological Subtype  |              |     |         |         |
| HR+ Her2–             | 162 (44.4%)  | 13  | 8%      | .022    |

2009. The patients had all undergone core biopsy for diagnosis along with ER, PR, and Her2 testing. They were staged according to American Joint Committee on Cancer Guidelines (AJCC), and the medical records were reviewed for clinical parameters and survival assessment.
went surgery either at the end of neoadjuvant chemotherapy (203/365-55.6%) or as an interval procedure (162/365-44.4%). The surgery done was mastectomy or breast-conserving surgery, and the axilla was treated by either axillary dissection or sentinel lymph node dissection. Radiotherapy was administered after the completion of chemotherapy and surgery. Adjuvant hormonal therapy was either with tamoxifen or with aromatase inhibitors (letrozole or anastrozole).

Assessment of response
The absence of invasive carcinoma in both breast and axillary lymph nodes in the post-surgery specimen was taken as pCR. Residual ductal carcinoma-in-situ was included in the pCR category. The post-neoadjuvant chemotherapy pathological stage, Tumor (T) and Nodal (N) statuses according to AJCC were among the factors that were studied in relation to DFS and OS.3

Statistical analysis
A comparison between various parameters and pCR was done using chi-square test and Fisher exact statistical tests, whereas Kaplan–Meier method was used to perform the analysis of survival in various subgroups. The log-rank or Breslow statistic was used for the univariate comparisons of median survival and the rate at specific time endpoints (with a 95% CI). Hazard ratio for survival with respect to various parameters was done by Cox regression analysis. A P value of <.05 was taken as significant. All analyses were carried out using the IBM-SPSS statistical software (version 20, International Business machines Corporation, USA). The median time period of follow-up was 49 months (7-163 months). DFS was measured from the date of first diagnosis to the date of first local or distant metastasis or last follow-up. OS was defined as the date of first diagnosis to death from any cause. Surviving patients without recurrence were censored at the date of last follow-up.

RESULTS

Patient characteristics
A total of 365 patients were analyzed retrospectively. The median age of our cohort was 50 years. There were a larger number of premenopausal patients (64.9%) and majority (83.5%) were stage III. HR positivity was seen in 58.6%, and 40.8% had a high-grade tumor. Our population had a higher number of triple negative tumors (21.3%) than most other series. The details of various characteristics and pCR rates in each subgroup are shown in Table 1.

Pathological complete response rates
The overall pCR was 13.7%. The achievement of pCR was not significant with respect to the age, menstrual

| Table 1 (cont.). Patient characteristics and pCR. |
|--------------------------------------------------|
| HR+ Her2+                                   | 47 (12.9%) | 9 | 19.1% |
| HR– Her2+                                   | 55 (15%)  | 13 | 23.6% |
| HR– Her2–                                   | 78 (21.3%) | 13 | 16.6% |
| HR+ Her2 unknown                            | 13 (3.6%)  | 0 | 0%    |
| HR– Her2 unknown                            | 10 (2.8%)  | 2 | 20%   |

Surgical type

| Surgical type        | No. (%) | DFS | P value |
|----------------------|---------|-----|---------|
| Mastectomy           | 296 (81%) | 40 | 13.5%   | .831 |
| Wide excision        | 69 (19%)  | 10 | 14.4%   |

Figure 1. A) Kaplan Meier curves showing OS with respect to pCR, B) yp stage and C) hormone receptor status.
status, and initial clinical stage of diagnosis or clinical presence of lymph nodes. Higher pCRs were however seen in inflammatory breast cancer. The most important factors associated with pCR were the biological factors. HR negative tumors had a consistently higher pCR (18.5%) as against HR positive tumors. Similarly Her2 positive tumors had a significantly higher pCR (21.3%) as against Her2 negative tumors. A total of 42.7% patients in the Her2 positive category received trastuzumab accounting for the high pCR rates. The highest pCR in our analysis of pathological subtypes was seen in the HR+, Her2neu+ and HR−, Her2neu+ groups at 19.1% and 23.6%, respectively. The triple negative subtype had a pCR of 16.6%, and the lowest rates were seen in the HR+, Her2neu− group. A total of 18.7% of grade III tumors had pCR as against 0% in grade 1 tumors.

Survival analysis
The median DFS in our population was 103.7 months. The 5-year DFS and OS were 58.9% and 66.5%, respectively.

The DFS and OS of patients achieving pCR were
significantly higher than those of patients with no pCR, and the advantage was continuing after more than 10 years of follow-up. Tumor stage, nodal stage, and pathological stage after neoadjuvant chemotherapy was significantly associated with prognosis \((P<.001)\) with worst outcomes for yp T3 T4, yp N2, N3, and yp stage III. The Kaplan–Meier estimates for DFS and OS at 5 years and 10 years are shown in Table 2 and Figure 1, respectively. Table 3 shows hazard ratio for DFS and OS in various categories by Cox regression analysis. Hazard ratio for DFS and OS were higher in grade 3 tumors as against grade 1 and 2 tumors. The most important pathological factor was hormone positivity, with hormone positive tumors showing higher DFS and OS as against negative tumors.

Prognostic significance of pathological subtype and their relation to pCR

The 5 year DFS and OS was 68.4% and 73.7% in the HR+Her2–, 54.9% and 74.8% for HR+Her2+, 52.9% and 64.6% for HR–Her2+ and 47.7% and 52.7% for triple negative (Figure 2). Cox regression analysis was done to know how the achievement of pCR affected the DFS and OS in various pathological parameters (Table 4). HR negative tumors who had achieved pCR had a better DFS as against those who had not which was not seen for the HR positive cohorts. Kaplan–Meier curves for the same are shown in Figure 3. Similarly there was a trend towards better DFS in HR–, Her2 positive patients who achieved pCR which was not seen in the other pathological subtypes. Kaplan–Meier survival curves for DFS and OS according to pCR rates for each pathological subtype are shown in Figure 4.

In the subgroup analysis of patients who had achieved pCR, triple negative patients did much worse than all other subgroups (Table 5).

DISCUSSION

Neoadjuvant chemotherapy results in equivalent efficacy and increased breast conservation as compared to standard adjuvant therapy. Use of primary systemic treatment allows for an in vivo assessment of chemotherapy sensitivity, based on the endpoint of pCR.

Our retrospective analysis was aimed at determining the pCR rates and their significance in a Middle Eastern population. Our cohort consisted of more triple negative patients and younger premenopausal patients as compared to other studies. Molecular subtypes of human breast cancer were first described by Perou et al in 2000. In clinical practice, IHC is used to approximate biological subtypes as follows: HR+Her2–, HR+Her2+, HR–Her2+ and Triple negative [HR–Her2–].

pCR has been shown to improve long-term survival and has been accepted as a surrogate endpoint. In our population, the overall pCR rates were 13.7%. This is lower than those reported from the western population in spite of having higher percentage of triple negative patients. Patients who achieved pCR in our study had a better 10 year DFS and OS.

In addition to pCR, HR status and histopathological subtype, the pathological stage after neoadjuvant chemotherapy also affected the DFS and OS, which is similar to previously reported. Post neoadjuvant T and N status also maintained their prognostic significance. In our analysis, HR positivity has more significance irrespective of Her2 status. HR+Her2+/ patients had better survival than both HR–Her2+ and triple negative patients. Hazard ratios for HR+Her2- and HR+Her2+ patients were equal for OS. Darb Esfahani et al has previously reported a 3-year survival of 90% for HR+ tumors irrespective of Her2 status as against 33% in HR–Her2+ and 65% in Triple negative tumors.
Table 3. Cox’s Regression analysis showing hazard ratio (HR) for various parameters with respect to DFS and OS.

| Parameter                        | DFS HR (95%CI) | P value | OS HR (95%CI) | P value |
|----------------------------------|----------------|---------|---------------|---------|
| Complete pathological response   |                |         |               |         |
| No pCR                           | 2.043 [1.10-3.77] | .023    | 1.884 [1.05-3.12] | .05     |
| yp T Status                      |                |         |               |         |
| T0/Tis                           | Reference      |         | Reference     |         |
| T1/Tmic                          | 1.472 [0.85-2.52] | .161    | 1.085 [0.62-1.89] | .774    |
| T2                               | 2.001 [1.175-3.40] | .011    | 1.696 [0.99-2.89] | .053    |
| T3/T4                            | 2.624 [1.512-5.27] | .001    | 2.852 [1.55-5.233] | .001    |
| yp N status                      |                |         |               |         |
| N0                               | Reference      |         | Reference     |         |
| N1                               | 1.727 [1.05-2.62] | .030    | 1.570 [0.93-2.62] | .085    |
| N2/3                             | 4.041 [2.69-6.28] | .000    | 3.391 [2.16-5.32] | .000    |
| yp Stage                         |                |         |               |         |
| Stage 0                          | Reference      |         | Reference     |         |
| Stage I                          | 0.763 [0.32-1.79] | .536    | 0.575 [0.23-1.43] | .234    |
| Stage IIA                        | 1.293 [0.62-2.66] | .487    | 1.148 [0.55-2.38] | .712    |
| Stage IIB                        | 1.690 [0.79-3.57] | .171    | 1.333 [0.61-2.87] | .462    |
| Stage III                        | 3.399 [1.81-6.37] | .000    | 2.614 [1.39-4.99] | .003    |
| Grade III vs. I/II               | 1.346 [0.952-1.904] | .093    | 1.573 [1.101-2.247] | .013    |
| HR positivity                    | 0.675 [1.089-2.056] | .018    | 0.575 [0.412-0.8084] | .001    |
| Her2 positivity                  | 1.221 [0.850-1.754] | .281    | 0.492 [0.583-1.295] | .492    |
| Pathology type                   |                |         |               |         |
| HR+Her2+                         | Reference      |         | Reference     |         |
| HR+Her2+                         | 1.496 [0.9-2.486] | .120    | 1 [0.553-1.807] | .10     |
| HR-Her 2+                        | 1.675 [1.024-2.74] | .040    | 1.357 [0.8-2.30] | .257    |
| Triple Neg.                      | 2.014 [1.32-3.075] | .001    | 2.265 [1.496-3.43] | .000    |

In our study, we could make out the paradoxes that well differentiated and HR positive tumors had a better long-term survival in spite of having lower pCR. Luminal A subtype also had a better OS than triple negative subtype in spite of having lower pCR with neoadjuvant chemotherapy. These findings have been previously reported from various other studies. von Michkwitz et al has reported that subgroups considered to have slowly proliferating tumors, pCR is not associated with prognosis, whereas in subgroups with highly proliferating tumors, pCR can discriminate between patients with good and poor prognosis accurately. The prognostic impact of pCR is highest in Her2-positive (non-luminal) and TN tumors, where patients achieving pCR show a prognosis comparable to that of patients with luminal A tumors.

Thus pCR is a strong prognostic factor in patients with HR negative tumors. In our analysis, HR negative patients achieving pCR had a statistically significant better DFS (P=.05). When DFS was analyzed according to pCR for biological subtypes, only the HR–Her2+ subtype was nearing significance. Patients with hormone positive subtypes should thus not be included in clinical trials where pCR is the endpoint.

Our analysis has significant drawbacks in spite of being the largest from the Middle East. It is a retrospective study done over a prolonged period of time. During this period significant change in the treatment of breast cancer has occurred including the approval of trastuzumab in the neoadjuvant setting. Also certain subsets of patients had very small numbers, making comparisons difficult.

In conclusion, this analysis of 365 women treated in Kuwait, confirms the prognostic value of pCR. HR status, tumor grade, and histopathological subtype are more important in determining a long-term survival. pCR as an endpoint for survival is thus more important in HR negative tumors.
### Table 4. Cox Regression analysis showing DFS and OS with respect to Complete pathological response for various biological parameters.

| Parameter | DFS | OS |
|-----------|-----|----|
|           | HR (95%CI) | P value | HR (95%CI) | P value |
| HR+       | 0.463 [0.169-1.267] | .134 | 0.658 [0.239-1.811] | .417 |
| HR–       | 0.468 [0.214-1.026] | .05  | 0.486 [0.222-1.062] | .071 |
| Her2+     | 0.266 [0.082-0.882] | .027 | 0.470 [0.142-1.557] | .217 |
| Her2–     | 0.635 [0.293-1.375] | .250 | 0.690 [0.319-1.494] | .347 |

**Pathological subtypes**

| HR+Her2– | 0.620 [0.193-1.988] | .421 | 0.728 [0.228-2.34] | .594 |
| HR+Her2+ | 0.237 [0.031-1.786] | .162 | 0.475 [0.06-3.757] | .481 |
| HR-Her2+ | 0.278 [0.065-1.191] | .085 | 0.42 [0.96-1.829] | .248 |
| Triple negative | 0.531 [0.188-1.501] | .232 | 0.511 [0.182-1.437] | .203 |

Note: All values are for patients who have achieved pCR to those who have not.

### Table 5. Kaplan Meier estimates for DFS and OS at 4 years in patients who have achieved pCR (N=48).

| Histological subtype | Patients (N) | Events DFS | DFS % | 95% CI | Events OS | OS % | 95% CI |
|----------------------|--------------|------------|-------|--------|-----------|-------|--------|
| HR+Her2–             | 13           | 3          | 84.6  | 64.6-100 | 3         | 83.3  | 62.2-100 |
| HR+Her2+             | 9            | 1          | 88.9  | 67.9-100 | 1         | 85.7  | 59.3-100 |
| HR-Her2+             | 13           | 2          | 84.6  | 64.6-100 | 2         | 84.6  | 64.6-100 |
| Triple Neg           | 13           | 4          | 69.2  | 43.6-94.8| 4         | 67.3  | 39.7-94.9 |

**Figure 4.** DFS for each pathological subtype with respect to pCR.
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