



Experience in the neoadjuvant treatment of triple-negative breast cancer at the University Hospital for Tumors

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Summary

Triple-negative breast cancer (TNBC) is a subtype of breast cancer with aggressive biological behavior and limited therapeutic options compared with other subtypes. Outcomes have improved following the introduction of immunotherapy. This retrospective study analyzed 45 patients with early triple-negative breast cancer from Croatia. They were treated at the University Hospital for Tumors, Sestre milosrdnice University Hospital Center. Patients received neoadjuvant therapy as per the KEYNOTE-522 study, which includes pembrolizumab with chemotherapy, followed by surgery. A pathological complete response (pCR) was achieved in 57.7% of patients. Complete radiomorphological regression on MRI was linked with a high pCR rate (94.1%). The phi correlation coefficient was 0.513, showing a moderate positive association between imaging and pathological response. A BRCA mutation rate of 22% supports routine genetic testing in TNBC patients. The results confirm the effectiveness of neoadjuvant immunochemotherapy in TNBC in clinical practice.

KEYWORDS: *triple negative breast neoplasms, pembrolizumab, neoadjuvant therapy, pathological complete response, magnetic resonance imaging, BRCA 1 protein and BRCA 2 protein, treatment outcome*

INTRODUCTION

Triple-negative breast cancer (TNBC) represents the most aggressive and therapeutically most challenging subtype of breast cancer(1–4). TNBC is characterized by the absence of expression of estrogen (ER) and progesterone (PR) hormone receptors, as well as HER2 receptors at the immunohistochemical level, and by aggressive tumor biology with a high degree of tumor heterogeneity and a pronounced immunosuppressive tumor microenvironment (TME), which ultimately contributes to poorer clinical outcomes, faster disease progression, and limited therapeutic options with a consequently high risk of early recurrence(2,3). TNBC accounts for approximately 15%–20% of all breast cancers, yet contributes disproportionately to breast cancer-related morbidity and mortality. Moreover, a higher preva-

lence of triple-negative breast cancer has been observed among women younger than 40 years(4). Patients with early-stage TNBC breast cancer, stages II to III, corresponding to local to locoregional disease extent, represent a particularly high-risk group with high rates of recurrence and mortality despite intensive multimodal therapy(5,6). The KEYNOTE-522 study established a new standard of care for high-risk early triple-negative breast cancer by introducing pembrolizumab in combination with neoadjuvant chemotherapy, with continuation of pembrolizumab in the adjuvant setting after surgical intervention(5,6).

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MATERIALS AND METHODS

A retrospective analysis was conducted, including 45 patients with early and locally advanced triple-negative breast cancer treated at the University Hospital for Tumors, Sestre milosrdnice University Hospital Center, during the period from February 2024 to July 2025. All patients initially received neoadjuvant therapy with pembrolizumab in combination with chemotherapy according to the KEYNOTE-522 study protocol, followed by surgical resection.

Descriptive statistics were used to present the demographic, clinical, and histopathological characteristics of patients. Categorical variables are presented as absolute numbers (n) and percentages (%). Continuous variables are shown as medians and ranges. The association between complete radiomorphological tumor regression on MRI and pathological complete response (pCR) was analyzed using Fisher's exact test. The phi (φ) coefficient was used to assess the strength of this association. Statistical significance was set at $p < 0.05$. Statistical analysis was performed with appropriate software. The results of these analyses are presented in the following section.

RESULTS

Our cohort included 45 patients who underwent surgical treatment after completion of neoadjuvant therapy for early triple-negative breast cancer. The cohort comprised 44 women and 1 man. The median age at diagnosis was 52 years (range 30–76 years). All patients underwent baseline breast magnetic resonance imaging. The median tumor size was 3 cm (range 0.7–5.5 cm); in two cases, the primary tumor was not visible on breast MRI, and in both cases, pathologically altered, biopsy-proven metastatic lymph nodes were identified in the ipsilateral axilla.

In 43 patients (95%), the histological subtype was non-specific type (NST, *no special type*). Two patients (4.4%) had a lobular histological subtype; in one, an apocrine histological subtype was described, and in the other, the histological subtype of the tumor cell nucleus was not specified. A total of 10 patients (22.2%) had a multifocal (n = 4; 8.8%) or multicentric (n = 6; 13.3%) tumor process. Regarding HER2 protein expression in our cohort,

64.4% of patients (n = 29) had HER2 expression 0, 24.4% (n = 11) had expression 1+, and 11.1% (n = 5) had expression 2+, all of whom were confirmed to be HER2-negative by silver in situ hybridization. Cytologically or histopathologically confirmed axillary involvement was present in 53.3% of patients, and in one patient, it was highly suspicious on imaging but not cytologically confirmed. All patients were treated according to a protocol consisting of triweekly carboplatin (4 cycles) with weekly paclitaxel (12 cycles), followed by a combination of doxorubicin and cyclophosphamide for four cycles at three-week intervals. Pembrolizumab was administered every three weeks at a dose of 200 mg intravenously. In 73.3% of patients (n = 33), a regressive response of the primary tumor was observed on follow-up breast ultrasound performed after the first block of planned treatment (4 cycles of triweekly carboplatin with 12 cycles of paclitaxel). In 13.3% of cases (n = 6), the requested follow-up ultrasound of the breasts and axillae was not performed, and in one patient, complete regression of the breast lesion was observed with persistently ultrasound-visible pathologically altered lymph nodes in the ipsilateral axilla. One patient experienced tumor progression.

After neoadjuvant treatment and before surgery, all patients received a follow-up breast MRI. Over 82% of patients responded: 37.7% (n = 17) had complete radiomorphological regression, and 44.4% (n = 19) had partial radiomorphological regression. In 17.7% of cases, MRI assessment of response was unavailable due to incomplete radiological data. One patient progressed. Of those with complete radiomorphological regression, 94.1% (16 of 17) also had pathological complete response (pCR) on final histopathology. In one patient, no residual breast tumor was found, but one of five ipsilateral axillary lymph nodes was tumor-positive. Among 28 cases without complete radiomorphological regression on MRI, 12 (42.8%) achieved pCR. The phi coefficient ($\varphi = 0.513$) showed a moderate positive association between radiological regression and pCR. Fisher's exact test confirmed a significant difference ($p = 0.001$) between complete radiomorphological regression on MRI and pCR. Radical surgery was performed in 29 patients (64.4%); 7 (15%) underwent bilateral mastectomy. All seven had confirmed BRCA mutations. Three additional BRCA mutations were found (total n = 10, 22%). In 64.4% of cases, pa-

tients were BRCA wild-type. BRCA mutation analysis was pending in the remaining patients.

According to the final histopathological diagnosis, 57.7% of patients achieved a pathological complete response (pCR). All 10 patients with BRCA mutations achieved pCR, whereas in the BRCA wild-type group, pCR was achieved in 48.2% of patients. In 3 patients (6.6%), a response with minimal residual disease burden was achieved, as defined by the Residual Cancer Burden (RCB) index (RCB-I). Additionally, in 3 cases (6.6%), a response with moderate residual disease burden (RCB-II) was achieved, while in 4 patients (8.8%), the response was characterized by a high residual tumor burden (RCB-III). In one case, the final histopathological examination revealed a change in disease biology, confirming a non-luminal, HER2-positive carcinoma with a high residual tumor burden (RCB-III).

DISCUSSION AND CONCLUSION

Triple-negative breast cancer is the most aggressive subtype of breast cancer. It remains a significant treatment challenge, especially in early and locally advanced cases. Neoadjuvant chemotherapy with pembrolizumab is the current standard for early-stage TNBC (stages II and III). This marked the first approval of immunotherapy for early breast cancer. The interaction between chemotherapy and immunotherapy is complex and not fully understood. The favorable response of this highly immunogenic tumor subtype to immunotherapy is likely influenced by tumor-infiltrating lymphocytes, tumor mutational burden, and the specific tumor microenvironment.

Phase III KEYNOTE-522 study results demonstrated a statistically significant increase in the pathological complete response (pCR) rate (64.8% in the pembrolizumab group versus 51.2% in the control group) and improvement in event-free survival (EFS) at 60 months: 81.2% in the pembrolizumab group versus 72.2% in the control group (HR 0.65), compared with chemotherapy alone(6,7). Final overall survival (OS) results, with a median follow-up of 75.1 months (as of March 2024), showed an estimated 60-month OS of 86.6% (95% CI: 84.0–88.8) in the pembrolizumab group versus 81.7% (95% CI: 77.5–85.2) in the chemotherapy-only control group, indicating that inclusion

of pembrolizumab in the standard treatment protocol statistically significantly reduced the risk of death by 34% ($p = 0.002$; HR 0.66), with benefits observed across all subgroups, regardless of PD-L1 status, tumor size, or regional lymph node involvement(6).

Our data were obtained from a retrospective analysis of 45 patients treated at the University Hospital for Tumors, Sestre milosrdnice University Hospital Centre, between February 2024 and July 2025. Only patients who underwent surgical intervention following neoadjuvant therapy were included in the analysis. Several limitations affect our study, including the small sample size from a single center and its retrospective design, which limit the generalizability of the findings. Despite the relatively small cohort of 45 patients, the results show a pathological complete response in 57.7% of patients, supporting the importance of neoadjuvant pembrolizumab in treating this highly aggressive breast cancer subtype. Our findings are largely consistent with published results of this protocol in real-world clinical practice, although the observed pCR rate is slightly lower than that in the registration KEYNOTE-522 trial (64.8%)(7-9). This discrepancy can be attributed to the clearly defined inclusion criteria and careful patient selection in randomized clinical trials compared with the heterogeneous patient populations encountered in routine clinical practice.

In this study, breast and axillary ultrasound were used solely for early assessment of therapeutic response after the first neoadjuvant treatment block, while breast magnetic resonance imaging (MRI) was the sole method for final radiological assessment of treatment response and comparison with histopathological findings.

Although protocols for radiological monitoring during neoadjuvant breast cancer treatment vary between institutions, literature data indicate that MRI is a valuable tool for monitoring response to neoadjuvant therapy, particularly in the context of triple-negative and HER2-positive subtypes(10). A limitation of this study is the unavailability of MRI data for some patients, which is a consequence of the retrospective nature of the analysis and real-world clinical practice.

Technical limitations of MRI can reduce sensitivity in detecting tumor response due to reactive inflammation, the presence of sclerosis and

necrosis in the tumor microenvironment, and potential overestimation of tumor size due to the antiangiogenic effect of systemic therapy, which interferes with contrast kinetics(10). In our cohort, a moderate positive correlation ($\phi = 0.513$) was observed between complete radiological regression on breast MRI after neoadjuvant treatment and pathological complete response (pCR), consistent with the worldwide literature(11,12).

Although a statistically significant association was found between complete radiomorphological regression on MRI and achievement of pCR, these results should be interpreted with caution due to the limited sample size and the retrospective design of our analysis.

According to the literature, imaging plays an important role in predicting therapeutic response, and a moderate correlation has been reported between complete radiological regression and pathological complete response (pCR) after neoadjuvant treatment of triple-negative breast cancer, supporting the use of MRI for guiding further clinical decisions, such as assessing the extent of surgical intervention; however, additional analyses on larger cohorts are needed(11,12).

In our patient cohort, the frequency of BRCA mutations was 22%, which is consistent with the global literature(13), further emphasizing the importance of genetic testing in this specific subgroup. According to international guidelines, all women diagnosed with triple-negative breast cancer are recommended to undergo testing for germline BRCA mutations (14). In our cohort, all patients were referred for genetic counseling, and those who underwent bilateral mastectomy were confirmed to have BRCA mutations. Early knowledge of BRCA status in patients with early triple-negative breast cancer allows consideration of additional therapeutic modalities, including prophylactic surgery and the potential use of targeted systemic therapies in patients who do not achieve pCR(15,16).

Consistent with the real-world literature, BRCA-mutated patients in our cohort achieved higher rates of pathological complete response than the wild-type subgroup(9). Given the small number of BRCA-mutated patients in our cohort, these results should be interpreted with caution due to the possibility of selection bias. Further studies in larger patient populations are needed to explore

potential de-escalation strategies(17). In one case within our cohort, a change in biological characteristics was observed, with confirmed HER2 positivity in the final histopathological report. Due to the retrospective nature of the analysis, it was not possible to further evaluate the mechanism underlying this change.

In conclusion, in real-world clinical practice, the effectiveness of neoadjuvant chemoimmunotherapy aligns with data reported in the literature. Drawing definitive conclusions requires longer follow-up in a larger patient cohort, and, consistent with real-world evidence, careful patient selection for this treatment protocol is crucial. Although our data are limited by sample size and short follow-up, they provide additional confirmation of the effectiveness of neoadjuvant chemoimmunotherapy in a real-world clinical setting at a tertiary oncology center.

REFERENCES

1. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, et al. Recent advances in therapeutic strategies for triple-negative breast cancer. *J Hematol Oncol.* 2022;15(1):121. doi:10.1186/s13045-022-01341-0
2. Borri F, Granaglia A. Pathology of triple negative breast cancer. *Semin Cancer Biol.* 2021;72:136–145. doi:10.1016/j.semcancer.2020.06.005
3. Derakhshan F, Reis-Filho JS. Pathogenesis of triple-negative breast cancer. *Annu Rev Pathol.* 2022;17:181–204. doi:10.1146/annurev-pathol-042420-093238
4. Bou Zerdan M, Ghorayeb T, Saliba F, Allam S, Bou Zerdan M, et al. Triple negative breast cancer: updates on classification and treatment in 2021. *Cancers (Basel).* 2022;14(5):1253. doi:10.3390/cancers14051253
5. Keenan TE, Tolaney SM. Role of immunotherapy in triple-negative breast cancer. *J Natl Compr Canc Netw.* 2020;18(4):479–489. doi:10.6004/jnccn.2020.7554
6. Michaels E, Chen N, Nanda R. The role of immunotherapy in triple-negative breast cancer (TNBC). *Clin Breast Cancer.* 2024;24(4):263–270. doi:10.1016/j.clbc.2024.03.001
7. Schmid P, Cortes J, Dent R, McArthur H, Pusztai L, et al; KEYNOTE-522 Investigators. Overall survival with pembrolizumab in early-stage triple-negative breast cancer. *N Engl J Med.* 2024;391(21):1981–1991. doi:10.1056/NEJMoa2409932
8. Rached L, Peyre-Pradat F, Spotti M, Baldini C, Laparra A, Lambotte O, et al. Real-world safety and effectiveness of neoadjuvant chemotherapy combination with pembrolizumab in triple-negative breast cancer.

- ESMO Real World Data Digit Oncol. 2024;5:100061. doi:10.1016/j.esmrod.2024.100061
9. Daniels E, McKeon J, Halstead A, de Oliveira Machado C, Potter S, Strawson-Smith T, et al. UK real-world evidence on pembrolizumab and neoadjuvant chemotherapy for triple-negative breast cancer. ESMO Open. 2025;10:104758. doi:10.1016/j.esmoop.2025.104758
 10. Connors C, Valente SA, ElSherif A, Escobar P, Chichura A, Kopicky L, et al. Real-world outcomes with the KEYNOTE-522 regimen in early-stage triple-negative breast cancer. Ann Surg Oncol. 2024. doi:10.1245/s10434-024-16390-7
 11. Kuzmova M, Cullinane C, Rutherford C, McCartan D, Rothwell J, Evoy D, et al. The accuracy of MRI in detecting pathological complete response following neoadjuvant chemotherapy in different breast cancer subtypes. Surg Oncol. 2023;51:102011. doi:10.1016/j.sur- onc.2023.102011
 12. Morris EA, Comstock CE, Lee CH, et al. MRI for monitoring response to neoadjuvant chemotherapy in breast cancer: a consensus statement from the ACR. J Magn Reson Imaging. 2017;45(3):530–537.
 13. Yeh RW, Houvenaeghel G, Mathelin C, et al. MRI and pathologic response after neoadjuvant chemotherapy in triple-negative breast cancer. Cancer Imaging. 2021;21(1):44.
 14. Tung N, Lin NU, Kidd J, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J Clin Oncol. 2016;34(13):1460–1468.
 15. Pujol P, Barberis M, Beer P, Friedman E, Piulats JM, Capoluongo ED, et al. Clinical practice guidelines for BRCA1 and BRCA2 genetic testing. Eur J Cancer. 2021;146:30–47. doi:10.1016/j.ejca.2020.12.023

Sažetak

Iskustvo u neoadjuvantnom liječenju trostruko negativnog raka dojke u Klinici za tumore

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Trostruko negativni karcinom dojke (TNBC) podtip je raka dojke s agresivnim biološkim ponašanjem i ograničenim terapijskim mogućnostima u usporedbi s drugim podtipovima, uz unaprijedene ishode uvođenjem imunoterapije. Ova retrospektivna studija analizirala je 45 bolesnika s ranim trostruko negativnim rakom dojke iz Hrvatske, liječenih u Klinici za tumore KBC Sestre milosrdnice. Bolesnici su liječeni neoadjuvantnim protokolom prema studiji KEYNOTE-522, koji uključuje pembrolizumab u kombinaciji s kemoterapijom, nakon čega je uslijedio kirurški zahvat. Kompletan patološki odgovor (pCR) postignut je kod 57,7% bolesnika. Potpuna radiomorfološka regresija na MR-u bila je povezana s visokom stopom pCR-a (94,1%), uz phi koeficijent korelacije od 0,513, što upućuje na umjerenu pozitivnu povezanost između slikovnog i patološkog odgovora. Uočen je udio BRCA mutacija od 22%, što podupire važnost rutinskog genetskog testiranja u bolesnika s TNBC-om. Dobiveni rezultati dodatno potvrđuju učinkovitost neoadjuvantnog imunokemoterapijskog pristupa u liječenju TNBC-a u svakodnevnoj kliničkoj praksi.

KLJUČNE RIJEČI: *trostruko negativan rak dojke, pembrolizumab, neoadjuvantna terapija, patološki potpuni odgovor, magnetska rezonancija, protein BRCA1 i protein BRCA2, ishod liječenja*