

อุบัติการณ์ภาวะเม็ดเลือดขาวชนิดนิวโทรฟิลต่ำระดับ 4 ในช่วงในช่วงระยะเม็ดเลือดขาวต่ำสุดหลังได้รับยาเคมีบำบัดสูตร Doxorubicin และ Cyclophosphamide รอบแรกในผู้ป่วยมะเร็งเต้านม

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ที่มาของปัญหา

ภาวะเม็ดเลือดขาวชนิดนิวโทรฟิลต่ำระดับ 4 (Grade 4 neutropenia) เป็นภาวะแทรกซ้อนที่พบบ่อยจากการรักษาด้วยยาเคมีบำบัดสูตร Doxorubicin และ Cyclophosphamide (AC) อย่างไรก็ตามข้อมูลอุบัติการณ์ของภาวะดังกล่าวในช่วงที่ระดับเม็ดเลือดขาวลดต่ำสุด (nadir period) รวมถึงปัจจัยที่เกี่ยวข้องและผลกระทบทางคลินิกในเวชปฏิบัติจริงของประเทศไทยยังมีจำกัด

วัตถุประสงค์

เพื่อศึกษาอุบัติการณ์ของภาวะ Grade 4 neutropenia ในช่วง nadir period หลังได้รับยาเคมีบำบัดสูตร AC รอบแรก และศึกษาปัจจัยที่มีความสัมพันธ์กับการเกิด Grade 4 neutropenia ตลอดจนภาวะแทรกซ้อนทางโลหิตวิทยาและการจัดการทางคลินิกที่เกี่ยวข้อง
วิธีการศึกษา: การศึกษาย้อนหลังเชิงสังเกตในผู้ป่วยมะเร็งเต้านมที่ได้รับยา Doxorubicin ขนาด 60 มก./ตร.ม. ร่วมกับ Cyclophosphamide ขนาด 600 มก./ตร.ม. ณ โรงพยาบาลพญาพัฒน์มคุณ ระหว่างเดือนพฤษภาคม พ.ศ. 2566 - 2569 เก็บรวบรวมข้อมูลทางคลินิกและผลตรวจ CBC ประมาณวันที่ 14 หลังได้รับยาเคมีบำบัดรอบแรก เพื่อวิเคราะห์อุบัติการณ์และปัจจัยเสี่ยงของการเกิดภาวะ Grade 4 neutropenia ในช่วง nadir period โดยใช้การถดถอยโลจิสติกแบบตัวแปรเดียวและแบบพหุตัวแปร

ผลการศึกษา

ผู้ป่วยจำนวน 138 ราย พบ Grade 4 neutropenia จำนวน 68 ราย คิดเป็นร้อยละ 49.3 (95% CI, 40.7–57.9) และพบ neutropenia ทุกระดับจำนวน 128 ราย คิดเป็นร้อยละ 92.8 (95% CI, 87.1–96.5) ไม่พบภาวะไข้ร่วมกับเม็ดเลือดขาวชนิดนิวโทรฟิลต่ำที่มีการบันทึกไว้ และไม่พบการเข้ารับการรักษาในโรงพยาบาลจากภาวะแทรกซ้อนที่เกี่ยวข้องกับ neutropenia การวิเคราะห์แบบพหุตัวแปรพบว่า ค่า Absolute Neutrophil Count (ANC) ก่อนเริ่มการรักษามีความสัมพันธ์อย่างอิสระกับการเกิด Grade 4 neutropenia โดยทุกครั้งที่ค่า ANC ก่อนเริ่มการรักษาเพิ่มขึ้น 1,000 เซลล์/ไมโครลิตร มีโอกาสเกิด Grade 4 neutropenia ลดลงร้อยละ 24 (adjusted OR 0.760; 95% CI, 0.601–0.962; p = 0.022)

สรุป

ผู้ป่วยมะเร็งเต้านมที่ได้รับยาเคมีบำบัดสูตร AC เกือบครึ่งหนึ่งเกิด Grade 4 neutropenia ในช่วง nadir หลังได้รับยาเคมีบำบัดรอบแรก ค่า ANC ก่อนเริ่มการรักษามีความสัมพันธ์กับการเกิด Grade 4 neutropenia โดยผู้ที่มีค่า ANC สูงกว่ามีโอกาสเกิด Grade 4 neutropenia ต่ำกว่า อย่างไรก็ตาม ผลการศึกษานี้ยังไม่สามารถยืนยันว่า ANC ก่อนเริ่มการรักษาสามารถใช้เป็นเครื่องมือทำนายภาวะไข้ร่วมกับ neutropenia หรือภาวะแทรกซ้อนทางคลินิกที่สำคัญได้ การติดตาม CBC ในช่วง nadir และการประเมินความเสี่ยงเป็นรายบุคคลจึงยังมีความสำคัญ และควรมีการศึกษาในกลุ่มตัวอย่างขนาดใหญ่เพื่อยืนยันผลดังกล่าว

คำสำคัญ

มะเร็งเต้านม; Doxorubicin; Cyclophosphamide; ภาวะเม็ดเลือดขาวชนิดนิวโทรฟิลต่ำระดับรุนแรง; เคมีบำบัด

Incidence of Grade 4 Neutropenia During the Nadir Period After the First Cycle of Doxorubicin and Cyclophosphamide Chemotherapy in Patients with Breast Cancer

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ABSTRACT

BACKGROUND

Grade 4 neutropenia is a common hematologic toxicity associated with doxorubicin and cyclophosphamide (AC) chemotherapy. However, the incidence of Grade 4 neutropenia during the nadir period after the first cycle of AC chemotherapy in routine clinical practice in Thailand remains unclear.

OBJECTIVES

This study aimed to determine the incidence of Grade 4 neutropenia during the nadir period after the first cycle of AC chemotherapy and to identify factors associated with Grade 4 neutropenia. Secondary objectives were to describe other hematologic toxicities and subsequent chemotherapy management following the first cycle.

METHODS

This retrospective observational study included patients with breast cancer who received doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²) chemotherapy at Pattaya Bhattamakun Hospital between May 2023 and May 2026. Complete blood counts obtained approximately 14 days after the first chemotherapy cycle were reviewed to assess hematologic toxicity during the expected nadir period. No patients received primary prophylactic granulocyte colony-stimulating factor (G-CSF) before the first cycle. Factors associated with Grade 4 neutropenia were evaluated using univariable and multivariable logistic regression analyses.

RESULTS

A total of 138 patients were included. Grade 4 neutropenia occurred in 68 patients, corresponding

to an incidence of 49.3% (95% CI, 40.7–57.9%). Overall, neutropenia of any grade occurred in 128 patients (92.8%; 95% CI, 87.1–96.5%). Anemia of any grade occurred in 23 patients (16.7%), and thrombocytopenia occurred in 1 patient (0.7%). No documented febrile neutropenia or neutropenia-related hospitalization was observed. Subsequent chemotherapy dose reduction was required in 13 patients (9.4%), and G-CSF was administered after chemotherapy in 3 patients (2.2%). In the multivariable analysis, baseline ANC was significantly associated with Grade 4 neutropenia. For every 1,000 cells/μL increase in baseline ANC, the odds of developing Grade 4 neutropenia decreased by 24% (adjusted OR, 0.760; 95% CI, 0.601–0.962; $p = 0.022$).

CONCLUSIONS

Grade 4 neutropenia occurred in approximately half of patients during the nadir period after the first cycle of AC chemotherapy. Baseline ANC was significantly associated with Grade 4 neutropenia; However, its ability to predict clinically consequential outcomes or guide prophylactic G-CSF use could not be established in this study. Further prospective studies are warranted to validate this association and determine its clinical utility.

KEYWORDS

breast cancer; doxorubicin; cyclophosphamide; severe neutropenia; absolute neutrophil count; chemotherapy induced neutropenia

Introduction

Breast cancer is the most common malignancy among women and remains a major public health concern both globally and in Thailand.^{1,2} According to Thailand Cancer Statistics 2022, breast cancer accounted for 41.7% of all newly diagnosed cancers among women.² Treatment strategies include surgery, radiotherapy, chemotherapy, endocrine therapy, targeted therapy, and immunotherapy, with treatment selection depending on tumor stage, molecular subtype, and individual patient characteristics. Chemotherapy, important role in the management of breast cancer. It may be administered in the neoadjuvant setting to reduce tumor size before surgery, in the adjuvant setting to eradicate residual microscopic disease and reduce the risk of recurrence, or in patients with metastatic disease to control tumor progression and improve survival. Among the commonly used chemotherapy regimens, doxorubicin plus cyclophosphamide (AC) remains a standard treatment for both early-stage and locally advanced breast cancer.^{3,4}

Although the AC regimen is effective, it is associated with several adverse effects, including alopecia, nausea and vomiting, fatigue, oral mucositis, and myelosuppression. Chemotherapy-induced neutropenia is one of the most clinically significant toxicities because it may increase the risk of infection, hospitalization, chemotherapy dose reduction or delay, and in severe cases, treatment-related mortality. The severity of neutropenia is classified into four grades according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE).⁵

The reported incidence of severe neutropenia following AC chemotherapy varies considerably among studies. A Korean multicenter study reported an incidence of grade 4 neutropenia of 44.6%,⁶ whereas a retrospective study from Surin Hospital, Thailand, reported an incidence of 18%.⁷ Previous clinical trials have reported febrile neutropenia rates ranging from 6% to 7% in patients receiving AC-based chemotherapy.⁸⁻¹¹ In contrast, the GEPAR DUO trial reported a febrile neutropenia incidence of only 3.7% in patients receiving neoadjuvant AC chemotherapy.¹² These variations may reflect

differences in patient characteristics, supportive care, monitoring strategies, and study design.

Chemotherapy-induced neutropenia commonly develops approximately 10–14 days after chemotherapy administration, corresponding to the expected nadir period, and generally recovers before the next chemotherapy cycle.¹⁴ At Pattaya Bhattamakun Hospital, patients receiving the AC regimen are routinely scheduled for follow-up approximately two weeks after chemotherapy administration, including clinical assessment and complete blood count (CBC) monitoring.

Therefore, this retrospective study aimed to determine the incidence of Grade 4 neutropenia during the nadir period after the first cycle of AC chemotherapy in patients with breast cancer. We also evaluated other hematologic toxicities and subsequent chemotherapy management and explored factors associated with Grade 4 neutropenia.

Materials and Methods

This retrospective observational study included patients with histologically confirmed breast cancer who received doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²) chemotherapy at Pattaya Bhattamakun Hospital between May 2023 and May 2026

Patients were excluded if they had incomplete medical records, lacked histopathological confirmation of breast cancer, did not receive the AC regimen, or did not complete follow-up at our institution. (Figure 1)

The study protocol was approved by the Chonburi Provincial Public Health Office Ethics Committee (Approval No. CBO REC 2569-022). Given of the retrospective study design and the use of anonymized patient data, the requirement for written informed consent was waived.

The standard AC regimen consisted of doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m². Chemotherapy doses were calculated according to body surface area (BSA) using the Mosteller formula for all patients. Five patients received an initial 10% dose reduction from the standard AC dose because of advanced age (>75 years) and multiple comorbidities. These patients were not excluded from the study and were retained in the analysis.

Initial dose reduction was included as a potential covariate in the regression analysis.

None of the patients received primary prophylactic granulocyte colony-stimulating factor (G-CSF) before the first cycle of AC chemotherapy. G-CSF was administered only after chemotherapy in three patients according to clinical judgment.

Data Collection

Patient demographics, clinical characteristics, tumor characteristics, and pretreatment laboratory parameters were retrieved from electronic medical records. Baseline laboratory evaluations included complete blood count (CBC), blood urea nitrogen (BUN), serum creatinine, and liver function tests obtained before the first cycle of chemotherapy.

The primary outcome was Grade 4 neutropenia during the nadir period after the first cycle of AC chemotherapy. Grade 4 neutropenia was defined according to CTCAE version 4.02 as an ANC of <500 cells/ μL .

At Pattaya Bhattamakun Hospital, CBC is routinely performed approximately 14 days after the first cycle of AC chemotherapy to assess hematologic toxicity during the expected nadir period. The primary outcome assessment was restricted to the first chemotherapy cycle and therefore did not represent the cumulative incidence of neutropenia across subsequent cycles.

Hematologic toxicities, including neutropenia, anemia, and thrombocytopenia, were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.02. Severe neutropenia was defined as Grade 4 neutropenia according to CTCAE criteria. The occurrence of febrile neutropenia (FN), G-CSF administration, and chemotherapy dose reduction in subsequent cycles were also recorded.

Febrile neutropenia was identified based on documented clinical and laboratory findings in the medical records. Because of the retrospective single-center design, episodes of FN that occurred outside our institution and were not subsequently reported or documented in the medical records could not be completely excluded.

Sample Size Calculation

The sample size was calculated based on the reported incidence of severe chemotherapy induced neutropenia during the nadir period in patients receiving doxorubicin and cyclophosphamide (18.0–44.6%).^{6,7} Assuming a 95% confidence level with a margin of error of 8%, the minimum required sample size was 96 patients. To compensate for an anticipated 10% rate of incomplete data, the target sample size was increased to 107 patients.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics. Continuous variables are presented as mean $\pm$ standard deviation (SD) or median (interquartile range [IQR]), as appropriate. Categorical variables are summarized as frequencies, percentages, and corresponding 95% confidence intervals (CIs).

The primary outcome was the incidence of Grade 4 neutropenia during the nadir period following the first cycle of AC chemotherapy. Secondary outcomes included the incidences of febrile neutropenia and other hematologic toxicities as well as subsequent chemotherapy dose reduction and G-CSF administration.

Potential risk factors associated with Grade 4 neutropenia were initially evaluated using univariable logistic regression analysis. Variables were selected for multivariable analysis based on clinical relevance, previous literature, and biological plausibility, irrespective of statistical significance in the univariable analysis. Multivariable logistic regression was performed using a backward stepwise selection procedure. Results are reported as odds ratios (ORs) with corresponding 95% CIs and p-values. A two-sided p-value <0.05 was considered statistically significant.

For continuous predictors, particularly baseline ANC and serum albumin, the assumption of linearity in the logit was assessed using the Box–Tidwell approach. No statistically significant evidence of non-linearity was observed for baseline ANC ($p = 0.943$) or serum albumin ($p = 0.282$). Therefore, both variables were retained as continuous predictors in the multivariable logistic regression model.

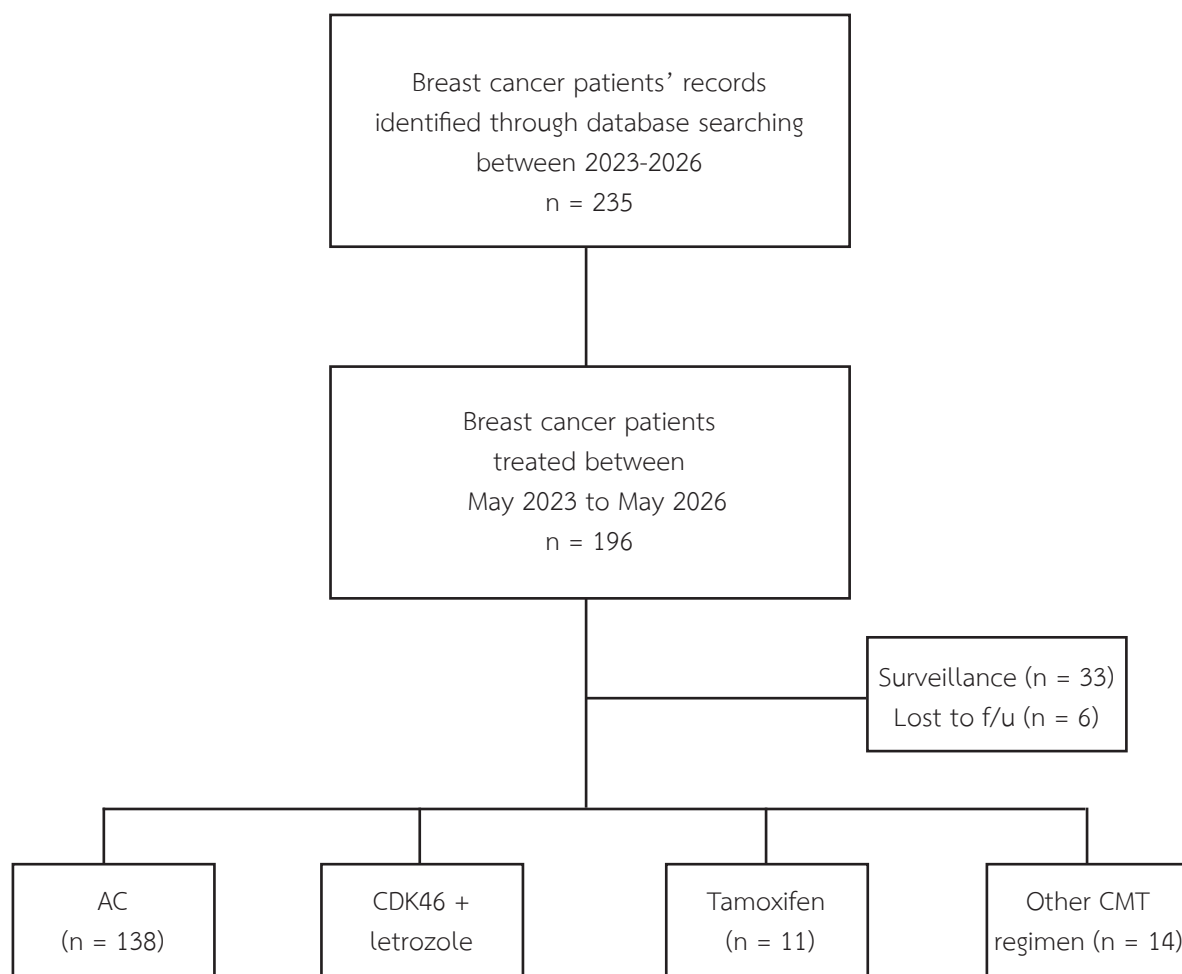


Figure 1. The consort diagram shows the patient inclusion and exclusion criteria. AC= Doxorubicin/Cyclophosphamide; CDK4/6 inhibitors = Ribociclib or Palbociclib; Other CMT = docetaxel, capecitabine, paclitaxel

Patient Characteristics

Between May 2023 and May 2026, 138 patients with breast cancer who received doxorubicin and cyclophosphamide (AC) chemotherapy at Pattaya Bhattamakun Hospital were included in the study. Baseline patient characteristics are summarized in Table 1.

The median age was 53 years (range, 31–78 years), and 18 patients (13.0%) were aged ≥ 65 years. The mean body weight and body surface area (BSA) were 59.9 kg and 1.62 m², respectively. Most patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 1 (97.1%). Regarding tumor subtype, 61 patients (44.9%) had basal-like breast cancer, while 40 patients (29.0%) had human epi-

dermal growth factor receptor 2 (HER2)-enriched disease. Forty-seven patients (34.1%) received neo-adjuvant chemotherapy, 78 (56.5%) received adjuvant chemotherapy, and 13 (9.4%) received palliative chemotherapy. (Table 1.)

Results

During the first cycle of AC chemotherapy, Grade 4 neutropenia during the expected nadir period occurred in 68 of 138 patients, corresponding to an incidence of 49.3% (95% CI, 40.7–57.9%) (Table 2). Overall, neutropenia of any grade occurred in 128 patients (92.8%; 95% CI, 87.1–96.5%). Grade 1–2 neutropenia was observed in 18 patients (13.0%; 95% CI, 7.9–19.8%), while Grade 3 neutropenia oc-

curred in 42 patients (30.4%; 95% CI, 22.9–38.8%). Anemia of any grade occurred in 23 patients (16.7%; 95% CI, 10.8–23.9%). Grade 1–2 anemia occurred in 21 patients (15.2%; 95% CI, 9.7–22.2%), and Grade 3 anemia occurred in 2 patients (1.4%; 95% CI, 0.2–5.1%). No patients developed Grade 4 anemia. Thrombocytopenia was uncommon, occurring in only one patient (0.7%; 95% CI, 0.0–4.0%), and no patients developed Grade 3 or higher thrombocytopenia. No documented episodes of febrile neutropenia were identified during the study period. No documented FN or neutropenia-related hospitalization was observed during the study period. Five patients (3.6%; 95% CI, 1.2–8.2%) received a 10% initial dose reduction because of advanced age (>75 years) and multiple comorbidities. None of the patients received primary prophylactic G-CSF during the first cycle of AC chemotherapy.

During subsequent cycles, chemotherapy dose reduction was required in 13 patients (9.4%; 95% CI, 5.1–15.6%), while G-CSF was administered to 3 patients (2.2%; 95% CI, 0.4–6.2%) following chemotherapy. Most patients completed subsequent chemotherapy without additional neutropenia-related interventions.

Univariable logistic regression analysis was performed to evaluate potential risk factors

associated with Grade 4 neutropenia. Variables including age, body mass index, ECOG performance status, menopausal status, baseline absolute neutrophil count (ANC), baseline serum albumin level, initial chemotherapy dose reduction, and other clinically relevant factors were evaluated.

None of the investigated variables demonstrated a statistically significant association with Grade 4 neutropenia in the univariable analysis at the 0.05 significance level.

Variables considered clinically relevant based on previous literature and biological plausibility were subsequently included in the multivariable logistic regression model to adjust for potential confounding factors. In the multivariable analysis, baseline ANC was independently associated with Grade 4 neutropenia. For every 1,000 cells/ μ L increase in baseline ANC, the odds of developing severe neutropenia decreased by 24% (adjusted OR, 0.760; 95% CI, 0.601–0.962; $p = 0.022$). No statistically significant evidence of non-linearity was observed for baseline ANC ($p = 0.943$) or serum albumin ($p = 0.282$). Age, menopausal status, initial chemotherapy dose reduction, and baseline serum albumin level were not significantly associated with severe neutropenia. (Table 3.)

Table 1. Baseline characteristics

Characteristics	Total (n = 138)	Characteristics	Total (n = 138)
Age –yr		cT–no. (%)	
Median (range)	53.23 (31-78)	1	9 (6.5)
Sex –no. (%)		2	75 (54.3)
Female	137 (99.3)	3	21 (15.2)
Male	1 (0.7)	4	33 (23.9)
Body weight (kg)	59.8 (41-97)	cN–no. (%)	
BMI		0	52 (37.7)
< 25 kg/m ²	74 (53.6)	1	57 (41.3)
≥ 25 kg/m ²	64 (46.4)	2	20 (14.5)
BSA (m²) (range)	1.62 (1.3-2.1)	3	9 (6.5)
ECOG performance statusscore –no. (%)		Ki 67 (range)	28.1 (1-85)
0-1	134 (97.1)	Histology –no. (%)	
2	4 (2.9)	Ductal carcinoma	131 (94.9)
Menopausal status –no. (%)		Lobular carcinoma	7 (5.1)
Premenopausal	66 (47.8)	Differentiated –no. (%)	
Postmenopausal	72 (52.2)	Welldifferentiated	3 (2.2)
Prior chemotherapy –no. (%)		Moderatelydifferentiated	66 (47.8)
Yes	1 (0.7)	Poorlydifferentiated	69 (50)
No	137 (99.3)	ER status –no. (%)	
Type of chemotherapy –no. (%)		Positive	77 (55.8)
NeoAdjuvant	47 (34.1)	Negative	61 (44.2)
Adjuvant	78 (56.5)	PR status –no. (%)	
Palliative	13 (9.4)	Positive	71 (51.4)
		Negative	67 (48.6)
		HER2 status –no. (%)	
		Positive	40 (29)
		Negative	86 (62.3)
		Equivocal	12 (8.7)

Table 2. Demonstrated the incidence of other hematologic toxicities in the nadir period,

Adverse events (N = 138)	no.(%)	95% CI
Neutropenia	128 (92.7)	87.1-96.5%
Grade 1-2	18 (13)	7.9-19.8%
Grade 3	42 (30.4)	22.9-39.8%
Grade 4	68 (49.3)	40.7-57.9%
Anemia	23 (16.7)	10.8-23.9%
Grade 1-2	21 (15.2)	9.7-22.2%
Grade 3	2 (1.4)	0.2-5.1%
Grade 4	0	0.0-2.6%
Thrombocytopenia	1 (0.7)	0.0-4.0%
Grade 1-2	1 (0.7)	0.0-4.0%
Grade 3	0	0.0-2.6%
Grade 4	0	0.0-2.6%
Febrile neutropenia	0	0.0-2.6%
Initial dose reduction	5 (3.6)	1.2-8.2%
Next cycle dose reduction	13 (9.4)	5.1-15.6%
*GCSF administration	3 (2.2)	0.4-6.2%

by using the **CTCAE grading system

*GCSF =granulocyte colony-stimulating factor

**CTCAE =Common Terminology Criteria for Adverse Events grading system

Neutropenia: Grade 1 (<LLN to 1,500/ μ L), Grade 2 (<1,500 to 1,000/ μ L), Grade 3 (<1,000 to 500/ μ L), and Grade 4 (<500/ μ L)

Anemia:Grade1(<LLNto10.0g/dL),Grade2(<10.0to8.0g/dL),Grade3(<8.0g/dL;ortransfusionindicated),andGrade4 (Life-threatening consequences)

Thrombocytopenia: Grade 1 (<LLN to 75,000/ μ L), Grade 2 (<75,000 to 50,000/ μ L), Grade 3 (<50,000 to 25,000/ μ L), and Grade 4 (<25,000/ μ L)

Table 3. Risk factor for severe neutropenia in multivariate analysis (multiple logistic regression, backward method).

Risk factor	Adjusted OR (95% CI)	p- value
Age	1.047 (0.984-1.114)	0.144
Menopause	0.483 (0.161-1.454)	0.196
Initial dose reduction	0.213 (0.018-2.496)	0.218
Initial Albumin	1.935 (0.524-7.149)	0.322
Initial ANC	0.760 (0.601-0.962)	0.022

ANC= absolute neutrophil count

Discussion

In this study, the incidence of Grade 4 neutropenia during the nadir period after the first cycle of doxorubicin and cyclophosphamide (AC) chemotherapy was 49.3% (95% CI, 40.7-57.9%), which is higher than the 18-44.6% incidence reported in previous studies.^{6,7} Several factors may explain this difference, including variations in patient characteristics, treatment setting, study design, supportive care, and neutrophil monitoring schedules. In particular, our study routinely assessed CBC approximately 14 days after chemotherapy, corresponding to the expected nadir period. This approach may have detected asymptomatic or clinically stable episodes of Grade 4 neutropenia that could have been missed in studies relying primarily on clinically indicated laboratory testing.

Despite the high incidence of Grade 4 neutropenia, no documented episodes of febrile neutropenia were identified. None of the patients received primary prophylactic G-CSF during the first cycle, thereby minimizing the potential influence of prophylactic G-CSF on

the observed incidence of neutropenia. Only three patients subsequently received G-CSF, and 9.4% required chemotherapy dose reduction in subsequent cycles.

The absence of documented febrile neutropenia may have several possible explanations. Routine laboratory monitoring may have facilitated early recognition of severe neutropenia before the development of infectious complications. In addition, the clinical significance of neutropenia depends not only on its severity but also on its duration, and the duration of neutropenia could not be determined in this study because CBC measurements were performed at predefined time points rather than continuously throughout the neutropenic period. Furthermore, although most patients underwent follow-up at our institution and laboratory results from other healthcare facilities were reviewed when available, episodes of febrile neutropenia treated at another hospital that were not subsequently reported or documented in our medical records could have been missed. Therefore, the absence of documented febrile neutropenia should be

interpreted with caution.

Importantly, the absence of febrile neutropenia does not diminish the clinical relevance of Grade 4 neutropenia. In our cohort, subsequent chemotherapy dose reduction occurred in 9.4% of patients, indicating that hematologic toxicity may influence subsequent treatment management. This finding highlights the potential impact of Grade 4 neutropenia on chemotherapy delivery and supports the importance of individualized risk assessment and appropriate supportive care. Patients treated in the early-stage setting may differ from those with metastatic disease in terms of treatment intent, baseline performance status, comorbidity burden, nutritional status, and organ function, all of which may influence chemotherapy tolerance and the risk of neutropenia. Nevertheless, despite the predominance of patients with early-stage disease and generally favorable baseline characteristics, the incidence of Grade 4 neutropenia remained high. This observation suggests that the myelosuppressive effect of the AC regimen and the timing of nadir assessment may have contributed substantially to the observed incidence. However, differences in patient populations and treatment settings should be considered when comparing our findings with previous studies.

In the multivariable logistic regression analysis, baseline absolute neutrophil count (ANC) was independently associated with Grade 4 neutropenia after adjustment for clinically relevant covariates. Specifically, a higher baseline ANC was associated with lower odds of developing Grade 4 neutropenia. This finding is biologically plausible because patients with a

lower baseline neutrophil reserve may be more susceptible to chemotherapy-induced bone marrow suppression. Baseline ANC is also readily available in routine clinical practice and may therefore have potential utility in individualized risk assessment before chemotherapy.

Although baseline ANC was statistically associated with Grade 4 neutropenia, the overall multivariable model did not reach statistical significance. Therefore, this finding should be interpreted as an association rather than evidence of a validated predictive model. The relatively small sample size may also have limited the ability of the multivariable analysis to identify additional independent predictors. Therefore, the observed association between baseline ANC and Grade 4 neutropenia should be interpreted cautiously and requires validation in larger prospective studies.

Limitations

Several limitations should be acknowledged. First, this was a retrospective study conducted at a single institution with a relatively small sample size. The study may therefore have limited statistical power to identify additional factors associated with Grade 4 neutropenia, and the findings may not be generalizable to other populations or treatment settings. Second, the study population was predominantly composed of patients with early-stage breast cancer, with most patients receiving neoadjuvant or adjuvant chemotherapy. This distribution may introduce selection bias and limits the generalizability of the findings to patients with metastatic breast cancer or other clinical populations. Differences

in treatment intent, baseline characteristics, comorbidity burden, and supportive-care practices across disease stages may also have influenced the observed incidence of Grade 4 neutropenia. Third, the primary outcome was assessed only during the nadir period after the first cycle of AC chemotherapy. Therefore, the incidence reported in this study should not be interpreted as the cumulative incidence of Grade 4 neutropenia over all chemotherapy cycles. Fourth, although CBC monitoring was routinely performed approximately 14 days after chemotherapy, the exact timing of blood sampling could vary among patients. Some patients also underwent CBC testing at other healthcare facilities. Such variation may have resulted in underestimation or overestimation of the incidence of Grade 4 neutropenia. In addition, a single nadir measurement did not allow assessment of the onset, duration, or recovery of neutropenia. Fifth, FN events managed at other healthcare facilities may not have been completely captured. Consequently, the finding of no documented FN should be interpreted cautiously.

Finally, secondary G-CSF administration and subsequent chemotherapy dose modifications were determined according to clinical judgment rather than a standardized protocol. This may have introduced variability in supportive-care management. Furthermore, because no patient received primary prophylactic G-CSF, this study

could not evaluate the effectiveness or clinical indications of primary G-CSF prophylaxis in patients receiving AC chemotherapy.

Conclusion

The incidence of Grade 4 neutropenia during the nadir period after the first cycle of AC chemotherapy was 49.3% in this cohort of patients with breast cancer. Baseline ANC was significantly associated with Grade 4 neutropenia, with higher baseline ANC associated with lower odds of developing Grade 4 neutropenia. However, no documented FN or neutropenia-related hospitalization was observed, and the present study cannot establish whether baseline ANC predicts clinically consequential outcomes or should be used to guide primary prophylactic G-CSF administration. Therefore, baseline ANC should be considered an associated clinical parameter rather than a validated predictive tool for clinically consequential neutropenia or FN.

The high incidence of Grade 4 neutropenia supports careful hematologic monitoring during the expected nadir period and individualized clinical assessment. Larger prospective studies are warranted to validate the association between baseline ANC and Grade 4 neutropenia and to determine whether baseline ANC can contribute to clinically meaningful risk stratification and supportive-care strategies.

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