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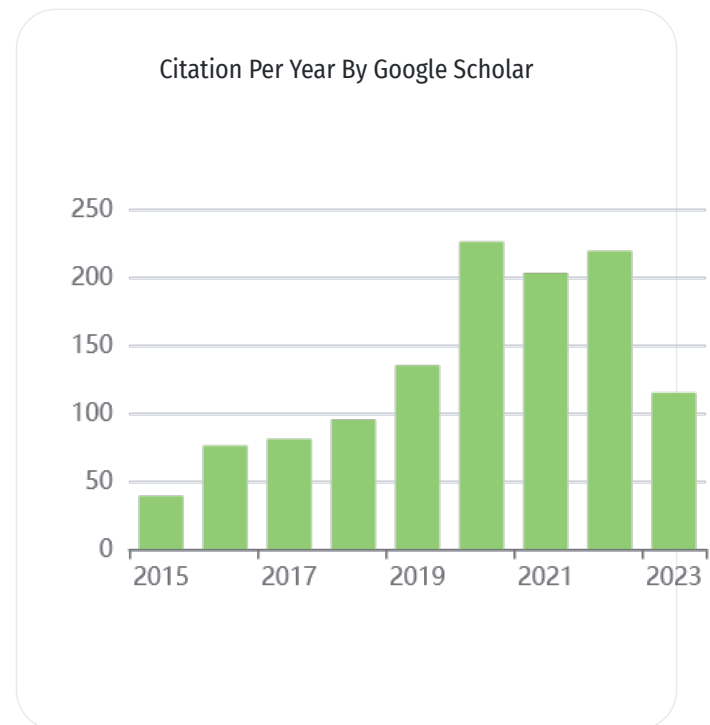
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ABSTRACT

The global incidence of testicular cancer is 1-2% from all cancers. The attempts to maintain high therapeutic rates while decreasing the treatment-related side effects and toxicity have become the current concern. However, the reports regarding testicular germ cell tumors (GCT) in Indonesia are limited. Thus, we aimed to evaluate the clinical characteristics of testicular GCT patients undergoing bleomycin, etoposide, and cisplatin (BEP) chemotherapy, as well as their chemotherapy response and side effects. We reported the data of patients with Testicular Germ Cell Tumor from January 2015 to December 2019. Several data were retrieved, including patient demographics, tumor characteristics, treatment, and prognosis outcome. A total of 67 patients with testicular germ cell tumors were included in this study. The mean age was 28.9 years old. The chemotherapy regimens used were four cycles of (BEP) in 36 patients (53.7%), followed by three cycles of BEP in 22 patients (32.8%). Patients with seminoma GCT mostly had a complete response (54.1%), whereas most patients with non-seminoma GCT had progressive disease (47.8%). The multiple logistic regression analysis showed that NLR and S staging were independently associated with the patient's response to chemotherapy (OR 2.14, 95% CI 1.22, 3.78, $p < 0.01$, OR 9.43, 95% CI 1.81, 49.14, $p < 0.01$). The clinical characteristics and response of testicular GCT patients among Indonesian men showed similarity with the current worldwide data. The NLR could be used as a potential biomarker for prognosis.

Keywords: Testicular cancer; germ cell tumor; BEP chemotherapy; cancer

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Hii j ni j tu

1. Neutrophil to lymphocyte ratio (NLR) has been reported by several studies for its role as a biomarker in various diseases, however, the role of NLR in testicular GCT is still unclear.
2. The characteristics and responses of testicular GCT patients among Indonesian men show similarity to other reports worldwide.
3. As a parameter, NLR shows promise to be used as a potential biomarker for prognosis in testicular GCT.

INTRODUCTION

Testicular cancer accounts for approximately 1-2% of all types of cancers globally (Purdue et al. 2005). They are considered rare tumors in the population as a whole, and most commonly found in males between 15 and 40 years old (Ghazarian et al. 2017). The incidence of testicular cancer has also increased in the Caucasian populations with a 7.8 and 6.7 in 100,000 men incidence rate in Western and Northern Europe, respectively, compared to Northern Africa which accounted for 0.6 in 100,000 men (Ferlay et al. 2010).

The most common type of testicular carcinoma was germ-cell tumors which accounted for 95% of all testicular carcinoma and further classified into seminomas and non-seminomatous GCS (NSGCT). Current treatment in testicular cancer was based on clinical staging from the International Germ Cell Cancer Collaborative Group (IGCCCG) for planning management strategies. The patients were classified for clinical stages based on radiology and tumor marker examination. The treatment options include surveillance, orchiectomy, chemotherapy based on the clinical stage of IGCCCG classification (Gaddam &

Chesnut 2021). The most used protocol regimen for chemotherapy was Bleomycin, Etoposide, and Cisplatin (BEP). BEP is still considered the best treatment in eligible patients. However, the adverse events might limit the beneficial effect of this regimen.

Despite having a great effectivity and high success rate, several side-effects were noted from BEP chemotherapy, including hypercholesterolemia, hypogonadism, and depression (Lavanderos et al. 2019). Attentions have increased lately on the effects of cancer treatment which attempts to maintain high therapeutic rates while decreasing the treatment-related side effects and toxicity. Treatment associated with side effects and toxicity has become an important issue for this population. Most existing articles reporting on GCT have been limited to a particular country or small groups within the country (Kusler & Poynter 2018).

Understanding the unique profile and response to chemotherapy in a population would allow an effective management strategy to further control and increase the survival rate (Farmanfarma et al. 2018). Identifying potential risk factors that may affect the patient's response to treatment is necessary to develop effective diagnostic and therapeutic strategies. The development of cancer progression was influenced by the tumor microenvironment and host inflammatory response. Several studies reported the neutrophil-to-lymphocyte ratio (NLR) for its role in numerous diseases and an increased ratio of NLR is a poor prognostic factor in testicular cancer (Ohno 2019, Mjaess et al. 2021). Its availability in urban and rural centers as well as its low price make it an appealing alternative for a prognostic biomarker in solid tumors (Zhang et al. 2017, Miyamoto et al. 2018, Prabawa et al. 2019, Yin et al. 2019). However, the evaluation of NLR use in GCT is limited. In Indonesia, reports regarding testicular GCT are highly limited. Thus, we aimed to summarize the clinical characteristics of testicular GCT patients undergoing BEP chemotherapy and analyze the prognostic factors affecting their response to chemotherapy.

MATERIALS AND METHODS

We conducted a retrospective analytical cohort study involving patients with testicular GCT treated with chemotherapy in an Indonesian tertiary hospital between January 2015 and December 2019. We include all patients diagnosed with Testicular Germ Cell Tumours (GCT) using clinical and pathological examination who received chemotherapy with or without surgical intervention. All patients who lost to follow-up and incomplete data were excluded from this study.

The medical records of the patient's visits, operations, and follow-up visits were reviewed retrospectively using the patient's unique hospital identification number. We extracted the data from medical records into a standardized data collection form comprised of patient's age, tumor stage from clinical and pathological examination, regimens and the cycle of chemotherapy, response to chemotherapy, and adverse events related to chemotherapy.

The tumor stage was classified using tumor-node-metastasis (TNM) classification according to the 7th edition of the American Joint Committee on Cancer (AJCC) 'cancer staging manual'. We evaluated serum tumor marker (S) staging by measuring lactate dehydrogenase (LDH), human chorionic gonadotropin (HCG), and alpha-fetoprotein (AFP). We classified the prognostic risk of the metastatic disease patients using the International Germ Cell Cancer Consensus Group (IGCCCG). The chemotherapy-related adverse events were assessed with a particular focus on the patient's symptoms, physical examinations, and routine blood counts and grouped using the classification of Common Terminology Criteria for Adverse Events (CTCAE) v5.0. In this study, we evaluated the patient's clinical response to chemotherapy using criteria, including complete response, partial response, stable disease, and progressive disease.

We summarized categorical data using frequency and percentage and displayed continuous data as mean and standard deviation (SD). All data were collected using spreadsheet software Microsoft excel® in 2021 (Microsoft Corporation, Redmond, WA, USA). In this study, we analyzed factors that were associated with patient's responses after receiving 3 or 4 cycles of chemotherapy. Bivariate analyses were performed using independent t-tests, Mann Whitney tests, and chi-square tests to analyze the differences between patients with complete and non-complete responses. We used multiple logistic regression analysis to find variables that were independently associated with patient's response to the chemotherapy. If the p-value of bivariate analysis was less than 0.25, we included the variables into the multiple logistic regression analysis. Observed associations were displayed as Odds Ratio (OR) with 95% Confidence Interval (95% CI). All analyses were conducted using statistical software SPSS ® 24 (IBM, Armonk, NY, USA).

The research related to human use in this study was performed in compliance with the ethical standards of the 1964 Helsinki Declaration, national regulations, and approved by the Research Ethical Committee, Faculty of Medicine, Universitas Airlangga No. 2000/109/II/2020.

RESULTS

Clinical characteristics

A total of 67 patients diagnosed with testicular GCT were included in this retrospective study. Table 1 showed the summary of clinical characteristics of the patients. The mean age was 28.9 years old, ranging from 8 months to 52 years old. The pathological findings showed that there were 40 patients (59.7%) presented with seminoma, and 27 patients (40.3%) presented with non-seminoma. There were 13 patients (19.4%) with Yolk sac pathology, 5 patients (7.5%) with embryonal carcinoma, and 5 patients (7.5%) with mixed GCT. Death was reported in a total of 9 patients, where 6 cases (9%) were caused by progressive disease and 3 cases (4.5%) were caused by chemotherapy adverse events (Table 1).

Tumor staging

The most frequently reported tumor stage was T2, which occurred in 28 patients (41.8%), followed by T4 in 5 patients (7.4%), and T3 in 22 patients (32.8%). Regarding nodal staging, N3 was the most commonly reported nodal stage with a total of 35 patients (52.2%). Metastatic disease (M1a/M1b) found in 52 patients (77.6%) (Table 1). From tumor marker staging, there were 19 patients (28.4%) with S1, 25 patients (37.3%) with S2, and 14 patients (20.9%) with S3. Based on the 2009 TNM substage classification, there were 8 patients (11.9%) with stage I, 3 patients (4.5%) with stage II, and 56 patients (83.6%) with stage III. According to the age group, seminoma GCT was predominantly presented at the age group of 30 to 39 years (65%), while non-seminoma GCT was primarily found in the age group of 0 to 9 years (40.3%).

Chemotherapy regimens

The most frequently used chemotherapy regimen was four cycles of bleomycin, etoposide, and Cisplatin (BEP), which were administered in 36 patients (53.7%), followed by three cycles of BEP in 22 patients (32.8%) (Table 1). Other chemotherapy regimens used in this study were four cycles of Etoposide and Cisplatin (EP) in 1 patient (1.5%) and one cycle of carboplatin in 1 patient (1.5%) (Table 1).

Chemotherapy adverse events

Table 1 displayed the details regarding the adverse events related to chemotherapy. Regarding adverse events evaluation, haematology and digestive systems were the most commonly affected systems occurred in 56 patients (83.6%) and 44 patients (66%), respectively. Neutropenia was the most frequent haematological adverse event in 42 patients (62.7%). In addition, severe nausea and vomiting requiring parenteral nutrition were reported in 4 patients (6%),

Table 1 displayed the details regarding the adverse events related to chemotherapy. Regarding adverse events evaluation, haematology and digestive systems were the most commonly affected systems occurred in 56 patients (83.6%) and 44 patients (66%), respectively. Neutropenia was the most frequent haematological adverse event in 42 patients (62.7%). In addition, severe nausea and vomiting requiring parenteral nutrition were reported in 4 patients (6%),

Table 1. Clinical characteristics of the patients with testicular GCT

Variables	n (%)
Age (mean ± SD)	28.9 ± 12.6
T classification	
T1/TX	12 (17.9%)
T2	28 (41.8%)
T3	22 (32.8%)
T4	5 (7.4%)
N classification	
N0/x	15 (22.4%)
N1	7 (10.4%)
N2	10 (14.9%)
N3	35 (52.2%)
M classification	
M0	15 (22.4%)
M1a/M1b	52 (77.6%)
S Classification	
S0	9 (13.4%)
S1	19 (28.4%)
S2	25 (37.3%)
S3	14 (20.9%)
Pathology	
Seminoma	40 (59.7%)
Non-Seminoma	27 (40.3%)
Embryonal Carcinoma	5 (7.5%)
Yolk Sac	13 (19.4%)
Mixed Germ Cell	5 (7.5%)
ICGGGC prognostic factor	
Good	27 (51.9%)
Intermediate	14 (26.9%)
Poor	11 (21.2%)
Pretreatment laboratory parameter	
Neutrophil (mean ± SD)	6.5 ± 3.68
Lymphocyte (mean ± SD)	2. ± 1.2
NLR (mean ± SD)	3.79 ± 2.16
UICC Stage	
IA/B/S	8 (11.9%)
IIC	3 (4.5%)
IIIA/B/C	56 (83.6%)
Chemotherapy regimens	
1 Cycle BEP	7 (11.9%)
3 Cycle BEP	22 (32.8%)
4 Cycle BEP	36 (53.7%)
4 Cycle EP	1 (1.5%)
1 Cycle Carboplatin	1 (1.5%)
Death	
Death due to progressive disease	6 (9%)
Death due to chemotherapy-related adverse events	3 (4.5%)

Clinical response

Table 2 showed the patients' clinical response to the chemotherapy. Based on the ICGGC prognostic risk classification, 14 patients (58.3%) with good prognostic risk responded completely to the chemotherapy. In the intermediate and poor-risk group,

Clinical response

Table 2 showed the patients' clinical response to the chemotherapy. Based on the ICGGC prognostic risk classification, 14 patients (58.3%) with good prognostic risk responded completely to the chemotherapy. In the intermediate and poor-risk group, most of the patients had progressive disease, which was occurred in 3 patients (25%) and 5 patients (55.6%) retrospectively. According to the pathological findings, patients with seminoma GCT mostly had a complete response (54.1%), whereas patients with non-seminoma GCT mostly had progressive disease (47.8%). Furthermore, NLR was observed to be higher in patients with stable disease (6±2.8) and progressive disease (5.46±1.88) compared to patients with complete and partial response (2.54±1.26 and 4.42±2.36, respectively).

Table 2. Frequency of patients with seminoma and non-seminoma GCT according to the age group

	Seminoma	Non-seminoma
Age (year)	n (%)	n (%)
Mean ± SD	34.5 ± 7.37	20.67 ± 14.38
Total	40 (59.7%)	27 (40.3%)
0-9	0 (0%)	9 (33.3%)
10-19	1 (2.5%)	2 (7.4%)
20-29	5 (12.5%)	9 (33.3%)
30-39	26 (65%)	4 (14.8%)
40-49	7 (17.5%)	3 (11.1%)
50-59	1 (2.5%)	0 (0%)

Factors associated with patient's response

The summary of analysis of factors associated with patients' responses was summarized in table 3 and 4.

Table 3. Summary of adverse events related to chemotherapy

Adverse events	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
Hematological system					
Thrombocytopenia	8 (72.7%)	2 (18.2%)	0 (0%)	1 (9.1%)	11 (16.4%)
Leukopenia	0 (0%)	2 (66.7%)	1 (33.3%)	0 (0%)	3 (4.5%)
Anemia	0 (0%)	9 (90%)	1 (10%)	0 (0%)	10 (14.9%)
Febrile Neutropenia	0 (0%)	0 (0%)	6 (100%)	0 (0%)	6 (9%)
GI system					
Nausea	2 (4.9%)	35 (85.4%)	4 (9.7%)	0 (0%)	41 (61.2%)
Vomiting	1 (6.2%)	11 (68.7%)	4 (25%)	0 (0%)	16 (23.8%)
Mucositis oral	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (1.5%)
Sepsis					5 (7.4%)

Based on the result of bivariate analysis, we found that NLR, age, M staging, S staging, and pathology had a significant association with patient's response (p < 0.25), and thus we include those variables in the multiple logistic regression analysis. The multiple logistic regression analysis, we discovered that NLR and S staging were independently associated with the patient's response to chemotherapy (OR 2.14, 95% CI 1.22, 3.78, p < 0.01; OR 9.43, 95% CI 1.81, 49.14, p < 0.01) (Table 4).

Table 4. Response to chemotherapy

	Complete n (%)	Partial n (%)	Stable n (%)	Progressive n (%)
ICGGC risk				
Good	14 (58.3%)	6 (25%)	0 (0%)	4 (16.7%)
Intermediate	2 (16.7%)	6 (50%)	1 (8.3%)	3 (25%)
poor	1 (11.1%)	2 (22.2%)	1 (11.1%)	5 (55.6%)
Pathology				
Seminoma	20 (54.1%)	12 (32.4%)	1 (2.7%)	4 (10.8%)
Non-seminoma	8 (34.8%)	3 (13%)	1 (4.3%)	11 (47.8%)
NLR (mean ± SD)	2.54 ± 1.26	4.42 ± 2.36	6 ± 2.83	5.46 ± 1.88

DISCUSSION

The incidence of testicular cancer peaks near birth, followed by a shallow rate before the second peak occurring safter puberty (Nistal et al. 2016). It follows a bimodal distribution with the initial peak happening before the age of four and the second peak occurring in the late 20s and early 30s (Steliarova-Foucher et al. 2017). The mean age of our patients was 29 years old, which was in line with global statistics.

The incidence rate among Asian men were ten times lower than Caucasian population (Park et al. 2018). However, most existing publications are limited to small groups in a few countries, making it difficult to compare the data with worldwide geographic patterns (Kusler & Poynter 2018). In a study which forecasted the future of testicular GCT incidence in the United States, the incidence in the Asian population was expected to rise, even though insignificantly (Ghazarian et al. 2017). Testicular GCTs develop from premalignant intratubular germ cells due to the failure of gonocytes maturation during fetal development. The progression toward invasive GCTS as seminoma or non-seminoma started after puberty (Batool et al. 2019). Seminoma and non-seminoma types make up almost 99% of all testicular GCTs (Farmanfarma et al. 2018).

The pathological findings of the patients showed that 40 patients (59.7%) presented with seminoma, and 27 patients (40.3%) presented with non-seminoma. These findings are similar to the distribution of types reported globally (Kusler and Poynter, 2018). Testicular germ cell tumors are believed to be chemosensitive with a high cure rate, even in a metastatic stage (Semaan et al. 2019).



In recent years, platinum -based chemotherapy has been given to improve the mortality rate of testicular GCT patients, with an overall current cure rate of more than 90% (Baroni et al. 2019). Current clinical data about BEP chemotherapy response in testicular cancer showed that BEP is the most effective combination regimen in treating disseminated non-seminomatous germ cell cancer. In this study, one patient did not receive Bleomycin because of poor pulmonary function.

Based on the recommendation reported by the European Association of Urology (EAU) guideline, one course of BEP regiment could be used in a patient who was unwilling to undergo surveillance, where efficacy had proven to be superior compared to RPLND. Patients undergoing surveillance could also undergo chemotherapy (Albers et al. 2015). The expected response rate was more than 90% in patients with a good prognosis, with few patients relapsing. Most patients in this study were given BEP, except for one patient who received Carboplatin. A total of 14 patients (58.3%) with reasonable prognostic risk had a complete response to the chemotherapy. In the intermediate and poor-risk group, most patients had progressive disease. Patients with seminoma GCT mostly had a complete response, whereas patients with non-seminoma GCT mostly had progressive disease. Although BEP chemotherapy produced excellent outcomes, it was associated with more toxicity, especially in pulmonary toxicity (den Hollander et al. 2016).

There was a 5% toxic-related death, sepsis or bleomycin-induced pneumonitis (Efstathiou & Logothetis 2006). There was a risk of leukemia associated with the amount of dose of chemotherapy (Howard et al. 2008). Cisplatin was associated with acute myeloid leukemia in a dose-dependent relationship. Hypogonadism and metabolic syndrome were also frequent in testicular cancer patients undergoing chemotherapy (Bogefors et al. 2017). A cardiovascular event incidence was also higher than the normal population (Lauritsen et al. 2020). In this study, hematological manifestation was the most common. A severe complication, such as sepsis, occurred in five patients, where three died due to septic shock and severe neutropenia.

Regarding response and prognosis, inflammation plays a role in the progressivity and prognosis of GCTs. One of the markers was NLR. Inflammatory cells produce mediators and cytokines that can induce or promote angiogenesis, tumor growth, invasion, and metastasis (Karakaya et al. 2021). Systemic inflammation has an essential role in all tumorigenesis stages. It may induce

the process via genetic mutations and genomic instability. Inflammation can also activate tissue repair, inducing the proliferation of premalignant cells (Grivennikov et al. 2010).

The current hypothesis states that the synthesis of inflammatory cytokines was affected by the tumor micro-environment, causing acute reactive changes in the neutrophil count (Tan et al. 2019, Iktac et al. 2020). Both neutrophils and lymphocytes are essential inflammatory mediators in many cancer types. Several studies suggested that neutrophil count supports tumor growth, while suppressing anti-tumor response (Fridlender & Albelda 2012). The activation of neutrophils might suppress lymphocyte function, causing immunosuppression and releasing enzymes with low anti-tumor activity (Gooden et al. 2011).

The parameter has been reported in other malignancies, such as breast cancer, in which several studies have used it to predict chemotherapy success (Chae et al. 2018). A study conducted by Hirahara et al. on gastric cancer patients concluded that the NLR above the cut-off point of 2.46 is associated with higher disease progressivity (Hirahara et al. 2019). A meta-analysis study concluded that NLR below the cut-off point of a specific population of patients was associated with a higher chance of complete response in solid tumor patients receiving chemotherapy (Li et al. 2018). Several studies are evaluating its role in germ cell tumors.

In this study, we observed a higher level of NLR in patients with stable disease (6 ± 2.8) and progressive disease (5.46 ± 1.88) compared to patients with complete and partial response (2.54 ± 1.26 and 4.42 ± 2.36 , respectively). After grouping the patients according to their response to the chemotherapy (complete and non-complete) in the bivariate analysis, we found that NLR, age, M staging, S staging, and tumor pathology had a significant association with the patient's response. Furthermore, from the result of the multiple logistic regression, we discovered that NLR was independently associated with the patient's response to the chemotherapy (OR 2.14, 95% CI 1.22, 3.78, $p < 0.01$). A similar finding also reported a correlation between high NLR value and low chemotherapy response rate (Karakaya et al. 2021). Survival parameters were reported that NLR was associated with progression-free survival (PFS) and overall survival (OS) (Ribnikar et al. 2021). Even though this study showed that NLR seemed to be higher in patients with stable and progressive disease than patients with partial and complete response, a definitive conclusion still could not be made until a prospective cohort prognostic study with larger sample size is performed.

Strength and limitation

This study had several limitations. It was conducted retrospectively in one center with a moderate sample size. Multiple future studies should be conducted in centers all over Indonesia to fully understand the demographic and clinical characteristics, and the chemotherapy response of Indonesian patients with testicular GCTs. A cohort analytical study analyzing the prognostic value of risk factors can be performed in a multicenter study with larger sample size.

CONCLUSION

The clinical characteristics and response of testicular GCT patients among Indonesian men showed similarity with current literature representing worldwide data. However, more extensive multicenter study is required to grasp the pattern of characteristics in Indonesian patients. The NLR results in this study indicated a potential biomarker as a poor prognostic factor in testicular GCT. However, further studies were required to fully determine its value.

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Conflict of interest

None0

Funding disclosure

Pone0

Author contribution

HMS and LH contributed conceptual, study design, collected and analysis data. HMS was write the manuscript. LH was checking grammar and validation of all manuscript data.

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