Effects of primary granulocyte-colony stimulating factor prophylaxis on the incidence of febrile neutropenia in patients with germ cell tumors

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Abstract. Testicular germ cell tumors (GCTs) are the most common solid malignancy in males aged 15-35 years. Febrile neutropenia (FN) is a serious complication of chemotherapy that frequently occurs in patients with GCTs. The present retrospective study aimed to evaluate the effect of primary granulocyte-colony stimulating factor (G-CSF) prophylaxis on the incidence of FN in patients with GCTs. The present study included a review of the medical records of patients diagnosed with GCTs treated with first-line/adjuvant chemotherapy between January 2000 and December 2017 at the National Cancer Institute (Bratislava, Slovakia). In January 2006, a decision was made to administer G-CSF prophylaxis (filgrastim or pegfilgrastim) to patients after every cycle of chemotherapy. The present study included 385 patients, and out of these, 264 patients received primary G-CSF prophylaxis, while 121 patients did not. A total of 71 patients (18.4%) suffered from FN events. In the subgroup that did not receive primary prophylaxis, 42 patients exhibited FN, while only 29 patients with primary prophylaxis suffered from FN (34.7 vs. 11.0%; P=0.00000003). According to the subgroup analysis, FN incidence was decreased in all groups that received primary prophylaxis, except for patients with stage I GCT receiving adjuvant chemotherapy, without affecting overall survival. Primary G-CSF prophylaxis was associated with markedly reduced FN incidence in patients treated with first-line chemotherapy for metastatic disease. Therefore, the results of the present study suggested that primary G-CSF prophylaxis should be considered in patients with GCT receiving first-line chemotherapy.

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Abbreviations: FN, febrile neutropenia; G-CSF, granulocyte-colony stimulating factor; GCTs, testicular germ cell tumors; IGCCCG, International Germ Cell Cancer Collaboration Group; NSGCT, non-seminomatous germ cell tumor; OS, overall survival; T-BEP, paclitaxel, bleomycin, etoposide and cisplatin; VIP, etoposide, iphosphamide and cisplatin

Key words: GCTs, FN, neutropenic fever, G-CSF, prophylaxis

Introduction

Testicular germ cell tumors (GCTs) account for only 1% of all solid tumors; however, they are the most common solid malignant tumors in males aged between 15 and 35 years (1,2). GCTs are considered a model for curable malignancies, as these tumors are exceedingly sensitive to cisplatin-based chemotherapy (3). However, chemotherapy regimens may induce non-negligible adverse effects (e.g., haematological, renal and gastrointestinal toxicity). Prevention and the correct management of treatment-related side effects are critical for minimizing morbidity and mortality, thus enhancing the quality of life of patients (4-6).

Febrile neutropenia (FN) is a life-threatening complication of cisplatin-dependent chemotherapy (7,8). Previous studies have demonstrated that the incidence of FN in patients with GCT varies greatly (9-12). The risk of developing FN depends on numerous factors associated with the patient and the treatment regimen. Previous studies have specified the potential risk factors associated with FN development in patients with GCT (9,12,13). Increased age has been recognized as a crucial risk factor for FN development, as Feldman *et al* (13) demonstrated a 44% incidence of FN in patients aged >50 years. Alternate risk factors observed by Terbuch *et al* (12) included a poor performance status [odds ratio (OR), 2.73; 95% confidence interval (CI), 1.47-5.06; P=0.001] and a poor-risk class according to the International Germ Cell Cancer Collaboration Group (IGCCCG) classification (14,15) (OR, 4.20; 95% CI, 1.71-10.33; P=0.002).

The use of prophylactic granulocyte-colony stimulating factor (G-CSF) following treatment with myelosuppressive chemotherapy has reduced the incidence of FN in various cancer types, including breast and small cell lung cancer (16).

Fossa *et al* (17) reported that prophylactic use of filgrastim in high-risk patients with GCT was associated with a decreased incidence of FN events. However, further studies assessing the effects of G-CSF prophylaxis on FN incidence in patients with GCT are required.

Therefore, the objective of the present retrospective study was to evaluate the effects of primary G-CSF prophylaxis on the incidence and outcomes of FN in patients with GCT.

Patients and methods

Study patients. The present retrospective study was conducted using the National Cancer Institute (Bratislava, Slovakia) medical record database of hospitalized patients with GCTs. The Institutional Review Board of the National Cancer Institute (Bratislava, Slovakia) approved the present study, and granted a waiver of consent (approval no. IZLO1). The subjects included patients diagnosed with GCTs, who were treated with first-line/adjuvant chemotherapy at the National Cancer Institute between January 2000 and December 2017. The present study excluded patients who had any concurrent malignancy, other than non-melanoma skin cancer, in the last 5 years. Patients who had undergone previous chemotherapy were also excluded. The study population consisted of male patients between 17 and 63 years of age (median, 31 years). Patients who did not receive G-CSF prophylaxis were aged between 17 and 63 years (median, 30 years). Patients who received G-CSF prophylaxis were aged between 18 and 61 years (median, 32 years). All patients received platinum-based chemotherapy regimens.

Definition of FN events. The European Society of Medical Oncology Clinical Practice Guidelines describe FN as a single oral temperature reading of >38.5˚C, or two consecutive readings of >38.0˚C for 2 h, combined with an absolute neutrophil count of $<0.5x10^9$/l, or a level anticipated to fall below $0.5x10^9$/l (18). While the baseline absolute neutrophil count is relatively stable in an individual, there is large interindividual variability. Therefore, the 'normal' range of absolute neutrophil count is very wide and is considered between $1.5x10^9$/l and $7x10^9$/l (19-21).

All FN episodes occurring during first-line chemotherapy were documented. Notably, only the first FN episodes that occurred in patients were deemed as events on the grounds that the aim of the present study was the assessment of the effects of primary G-CSF prophylaxis. Some patients who suffered FN events and did not receive primary G-CSF prophylaxis received secondary prophylaxis in subsequent chemotherapy cycles.

Baseline data. Patients underwent chest, abdominal and pelvic computed tomography scans during the initial staging. The baseline data included age, primary tumor location, tumor histology, TNM stage (22), IGCCCG risk class and first-line chemotherapy regimen.

FN prophylaxis. Only 1 of the patients treated before January 2006 received primary G-CSF prophylaxis. In January 2006, a decision was made by the National Cancer Institute to administer G-CSF prophylaxis (filgrastim or pegfilgrastim) to male GCT patients after every cycle of chemotherapy. Fig. 1 shows a flow diagram outlining the selection process of the study population and a brief timeline of G-CSF prophylaxis implementation. There was only 1 patient (0.9%) before January 2006, who received primary G-CSF prophylaxis. During the transition period in the years 2006 and 2007, 26 patients (72.2%) received primary G-CSF prophylaxis. From 2008, 237 patients (98.3%) received primary G-CSF prophylaxis.

Filgrastim (480 μg, subcutaneous) was administered for 10 days, on days 6, 7, 9-14, 16 and 17 in the bleomycin, etoposide and cisplatin (BEP) regimen, and for 10 consecutive days beginning on day 6 in all other regimens. Pegfilgrastim (6 mg/0.6 ml subcutaneous) was administered on day 6. Chemotherapy dosing schedules are shown in Table SI.

When FN was observed following the first chemotherapy cycle, and the patient did not receive primary G-CSF prophylaxis, G-CSF was administered in subsequent cycles.

Statistical analysis. The present study performed a retrospective review of the medical records of the patients. All first episodes of FN were categorized as events. Characteristics of patients are presented as the median (range) values for continuous variables and frequency (percentage) for categorical variables. Using Fisher's exact test for statistical analysis, FN events were compared between groups with and without prophylaxis. $P \leq 0.05$ was considered to indicate a statistically significant difference.

The primary outcome was the overall incidence of FN events in the first-line chemotherapy. The secondary outcomes included incidences of FN events in the numerous subgroups and overall survival (OS).

Median follow-up was defined as the median observation time of the patients. OS was determined using the chemotherapy start date, and the last follow-up date or death. The median follow-up time of all patients was 68 months (range, 0-224 months), while the median follow-up time of living patients was 82 months (range, 6-224 months) (Table I). The last follow-up date was May 15, 2019. Follow-up included history and physical examination, measurement of serum tumor markers and imaging studies of the chest, abdomen and pelvis. The follow-up frequency was guided by the initial tumor histology and clinical stage as recommended by the European Society of Medical Oncology clinical practice guidelines (23). Kaplan-Meier analysis was performed to calculate OS. The differences in survival between patients with and without prophylaxis were calculated using the log-rank test. A multivariate Cox proportional hazards model for OS revealed the differences in outcomes following G-CSF prophylaxis and prognosis, depending on age and IGCCCG risk score. NCSS 2019 statistical software was utilized for all statistical analyses (24).

Results

Patient characteristics. The cohort described in the present study included 385 chemotherapy-naive patients with GCTs, treated with first-line chemotherapy (Fig. 1). A summary of patient characteristics is shown in Table I. The median age

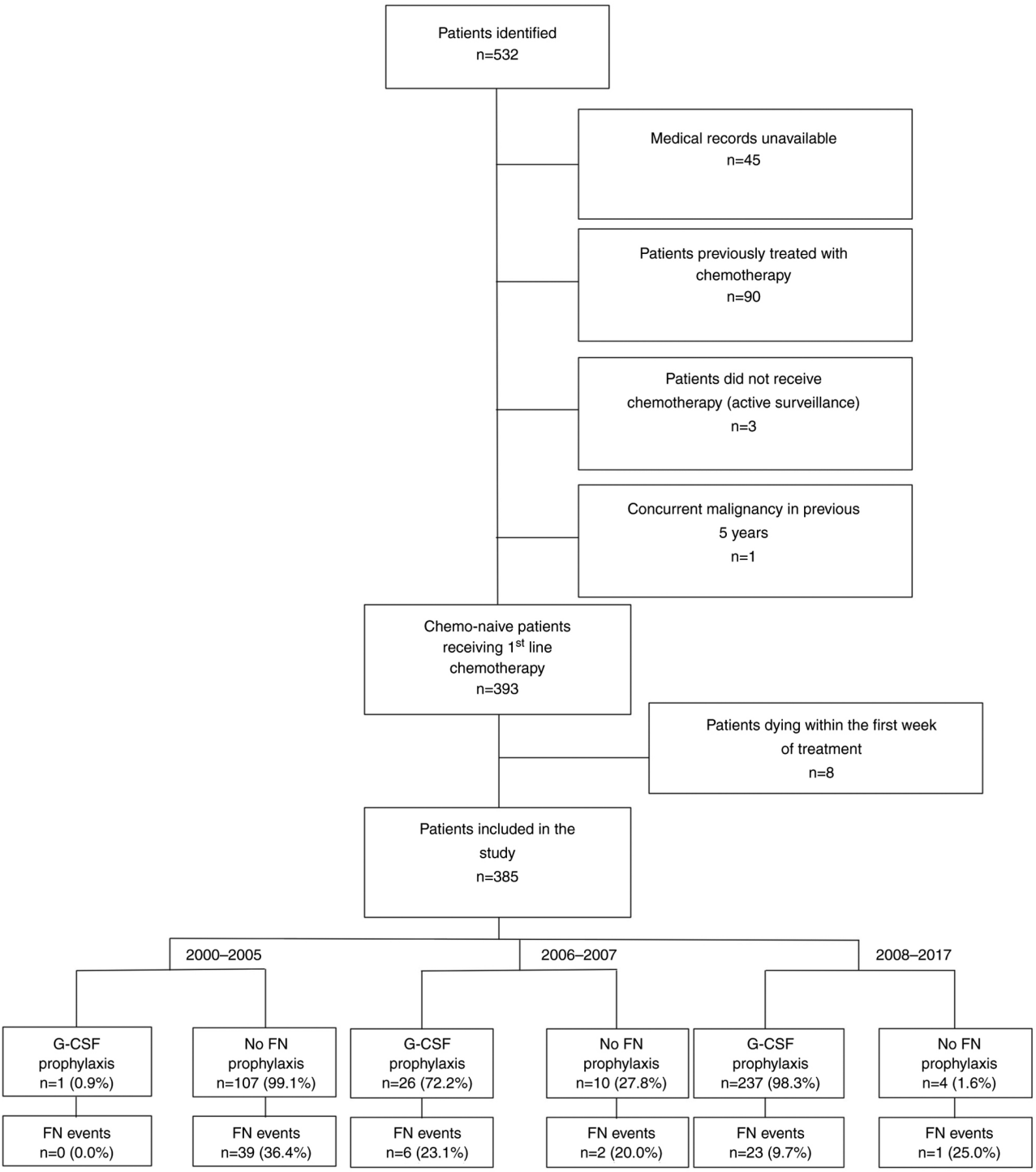

Figure 1. Flow diagram demonstrating the method used to search the medical records of patients with germ cell tumors treated between 2000 and 2017. FN, febrile neutropenia; G-CSF, granulocyte-colony stimulating factor.

of patients at the time of enrollment was 31 years (range, 17-63 years). The majority of patients (75.8%) exhibited non-seminomatous germ cell tumors (NSGCTs). All patients received platinum-based chemotherapy. A total of 121 patients (31.4%) did not receive primary G-CSF prophylaxis, and 264 (68.6%) received primary prophylaxis. A total of 39 of these patients (14.8%) received filgrastim, while 225 (85.2%) were administered pegfilgrastim. Out of 272 patients treated with BEP, 176 (64.7%) were administered pegfilgrastim (Table I).

FN events. During the study period, 71 patients (18.4%) suffered FN events. Of the 121 patients who did not receive primary prophylaxis, 42 (34.7%) suffered from FN events. Of these 121 patients, 31 patients (25.6%) had only 1 FN episode, and 11 patients (9.1%) experienced >1. Of the 264 patients receiving prophylaxis, 29 (11.0%) had FN events, 25 (9.5%) experienced only 1 FN episode and 4 patients (1.5%) had >1 episode (Table II).

The majority of FN episodes (69.1%) occurred in the first chemotherapy cycle. Overall, FN incidence after the

Table I. Patient characteristics (n=385).

Characteristics	Value
Histology, n (%)	
Seminoma	87 (22.6)
NSGCT	292 (75.8)
Unknown[a]	6 (1.6)
Primary tumor, n (%)	
Gonadal	344 (89.4)
Extragonadal	41 (10.6)
Retroperitoneum	25 (6.5)
Mediastinum	12 (3.1)
CNS	2 (0.5)
Unknown	2 (0.5)
Stage, n (%)	
I.A-B	39 (10.1)
I.S	22 (5.7)
II.A	31 (8.1)
II.B	48 (12.5)
II.C	47 (12.2)
III.A	49 (12.7)
III.B	50 (13.0)
III.C	99 (25.7)
IGCCCG risk group, n (%)	
Good	206 (53.5)
Intermediate	51 (13.2)
Poor	89 (23.1)
Treatment regimen, n (%)	
BEP	273 (70.9)
EP	51 (13.2)
Other regimen	61 (15.8)
Follow-up status, n (%)	
Alive	332 (86.2)
Dead	53 (13.8)
Median follow-up time (range), months	68 (0-224)
Median follow-up time for alive patients (range), months	82 (6-224)
Estimated 2-year OS rate, %	88.6
Estimated 5-year OS rate, %	84.8
Primary G-CSF prophylaxis, n (%)	
No prophylaxis	121
G-CSF prophylaxis	264
Filgrastim	39
Pegfligrastim	225

[a]Histological confirmation was not available at the time of initial treatment start date. Treatment was administered based on typical clinical presentation and high levels of serum tumor markers. NSGCT, non-seminomatous germ cell tumor; CNS, central nervous system; IGCCCG, International Germ Cell Cancer Collaborative Group; BEP, bleomycin, etoposide and cisplatin; EP, etoposide and cisplatin; OS, overall survival.

first chemotherapy cycle was 16.8%. In patients receiving prophylaxis, FN incidence after the first chemotherapy cycle was 9.8%, while FN incidence was 32.3% in those without prophylaxis (P<0.0001; data not shown). The FN incidence in patients administered filgrastim and pegfilgrastim was 20.5 and 9.3%, respectively (P=0.0393). Patients that received G-CSF prophylaxis experienced a prolonged period before experiencing FN compared with patients without prophylaxis [hazard ratio (HR), 0.30; 95% CI, 0.18-0.50; P=0.00000001]. A total of 11 (2.9%) patients died during the chemotherapy. Of these, 8 patients experienced FN events, with 4 receiving primary G-CSF prophylaxis and 4 patients not.

Association between FN prophylaxis and patient/tumor characteristics. The highest FN incidence (42.6%) occurred in patients that received a chemotherapy regimen other than BEP, or etoposide and cisplatin (EP). The two regimens most frequently associated with FN development were the paclitaxel, bleomycin, etoposide and cisplatin (T-BEP; 69.0%) regimen, and the etoposide, iphosphamide and cisplatin (VIP; 50.0%) regimen. A high FN incidence was also observed in patients with poor-risk disease according to the IGCCCG classification (41.6%), and in those with extragonadal tumors (29.3%).

A total of 61 FN events (20.9%) occurred in patients with NSGCTs and 10 (11.5%) in patients with seminoma. While there was a significantly lower (11.9 vs. 38.4%; P<0.0001) FN incidence in patients with NSGCT receiving prophylaxis compared with that in patients without prophylaxis, the difference in incidence rates in patients with seminoma was not statistically significant (9.2 vs. 18.2%; P=0.2552).

A total of 59 FN events (17.2%) were observed in patients with gonadal tumors, and 12 (29.3%) were recorded in patients with extragonadal tumors. There was a significantly lower FN incidence in patients receiving prophylaxis for both primary tumor locations (P<0.0001 and P=0.0011 for patients with gonadal and extragonadal tumors respectively).

A total of 22 (10.7%) FN events occurred in patients with good-risk disease, according to the IGCCCG classification, a total of 10 events (19.6%) occurred in those with intermediate-risk disease and 37 events (41.6%) occurred in patients with poor-risk disease. There was a significantly lower FN incidence in patients receiving prophylaxis with either good (P=0.0017) or poor-risk disease (P=0.0003). A lower incidence rate was observed in patients receiving G-CSF prophylaxis with intermediate-risk disease, but this difference was not statistically significant (P=0.0747). These data are summarized in Table II.

FN events occurred in 40 patients (14.7%) receiving BEP, 20 patients (69.0%) receiving T-BEP, 5 patients (9.8%) receiving EP, 4 patients (50.0%) receiving VIP and 2 patients (22.2%) receiving the paclitaxel, iphosphamide and cisplatin regimen. No FN events occurred in patients who received GETUG 13 (25) or other regimens (Table III).

A significantly lower (P=0.0296) FN incidence occurred in patients receiving G-CSF prophylaxis, according to a subgroup analysis of patients subjected to BEP chemotherapy compared with patients without prophylaxis. The incidence was also significantly lower in the BEP subgroup for patients receiving prophylaxis with NSGCT histology (P=0.0250) or good-risk disease (P=0.0061) in comparison with patients without

Table II. FN events.

Variables	Overall incidence, n (%)	Incidence in the no prophylaxis group, n (%)	Incidence in the G-CSF prophylaxis group, n (%)	P-value
All FN events	71/385 (18.4)	42/121 (34.7)	29/264 (11.0)	≤0.0001
Histology				
Seminoma	10/87 (11.5)	4/22 (18.2)	6/65 (9.2)	0.2552
NSGCT	61/292 (20.9)	38/99 (38.4)	23/193 (11.9)	≤0.0001
Primary tumor location				
Gonadal	59/344 (17.2)	32/103 (31.1)	27/241 (11.2)	≤0.0001
Extragonadal	12/41 (29.3)	10/18 (55.6)	2/23 (8.7)	0.0011
Stage IA/B	2/39 (5.1)	0/12 (0.0)	2/27 (7.4)	0.3330
IGCCCG risk group				
Good	22/206 (10.7)	12/55 (21.8)	10/151 (6.6)	0.0017
Intermediate	10/51 (19.6)	5/14 (35.7)	5/37 (13.5)	0.0747
Poor	37/89 (41.6)	25/40 (62.5)	12/49 (24.5)	0.0003
Chemotherapy regimen				
BEP	40/273 (14.7)	16/69 (23.2)	24/204 (11.8)	0.0204
EP	5/51 (9.8)	3/19 (15.8)	2/32 (6.3)	0.2680
Other	26/61 (42.6)	23/33 (69.7)	3/28 (10.7)	≤0.0001
FN episodes per patient				
1	56/385 (14.5)	31/121 (25.6)	25/264 (9.50)	
>1	15/385 (3.90)	11/121 (9.09)	4/264 (1.52)	

Data is presented as n/total n. FN, febrile neutropenia; G-CSF, granulocyte-colony stimulating factor; NSGCT, non-seminomatous germ cell tumor; IGCCCG, International Germ Cell Cancer Collaborative Group; BEP, bleomycin, etoposide and cisplatin; EP, etoposide and cisplatin.

Table III. FN event incidence based on chemotherapy regimen.

Regimen	Overall incidence, n (%)	Incidence in the no prophylaxis group, n (%)	Incidence in the G-CSF prophylaxis group, n (%)
BEP	40/273 (14.7)	16/69 (23.2)	24/204 (11.8)
EP	5/51 (9.8)	3/19 (15.8)	2/32 (6.3)
T-BEP	20/29 (69.0)	19/27 (70.4)	1/2 (50.0)
GETUG 13	0/11 (0.0)	0/0 (0.0)	0/11 (0.0)
TIP	2/9 (22.2)	0/0 (0.0)	2/9 (22.2)
VIP	4/8 (50.0)	4/6 (66.7)	0/2 (0.0)
Other[a]	0/4 (0.0)	0/0 (0.0)	0/4 (0.0)

[a]A single patient received 1st cycle chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine, prednisone due to initial suspicion of lymphoma, and was subsequently treated with 4 cycles of EP. Another 2 patients received BEP with carboplatin due to renal parameters and 1 patient with liver insufficiency due to cirrhosis was treated with cisplatin monotherapy. FN, febrile neutropenia; G-CSF, granulocyte-colony stimulating factor; BEP, bleomycin, etoposide and cisplatin; EP, etoposide and cisplatin; T-BEP, paclitaxel, bleomycin, etoposide and cisplatin; GETUG 13, dose dense regimen; TIP, paclitaxel, iphosphamide and cisplatin; VIP, etoposide, iphosphamide and cisplatin.

prophylaxis. A lower (P=0.0578) FN incidence occurred in patients with extragonadal tumors treated with BEP who received G-CSF prophylaxis compared with that in patients without prophylaxis. These results are shown in Table IV.

Association between OS and G-CSF prophylaxis. The median follow-up time of all patients was 68 months (range, 0-224 months), while the median follow-up time of living patients was 82 months (range, 6-224 months) (Table I). A total of 53 deaths (13.8%) occurred in the present study population (Table V). The estimated 2- and 5-year OS rates of the patients were 88.6 and 84.8%, respectively (Table VI).

Patients receiving G-CSF prophylaxis exhibited a significantly prolonged OS rate (HR, 0.54; 95% CI, 0.31-0.96; P=0.0235) compared with patients without it (Fig. 2). These

Table IV. FN events in patients receiving the bleomycin, etoposide and cisplatin regimen.

Variables	Overall incidence, n (%)	Incidence in the no prophylaxis group, n (%)	Incidence in the G-CSF prophylaxis group, n (%)	P-value
All FN events	40/272 (14.7)	16/69 (23.2)	24/203 (11.8)	0.0296
Histology[a]				
Seminoma	4/34 (11.8)	0/3 (0.0)	4/31 (12.9)	>0.999
NSGCT	36/237 (15.2)	16/66 (24.2)	20/171 (11.7)	0.0250
Primary tumor location				
Gonadal	35/252 (13.9)	12/61 (19.7)	23/191 (12.0)	0.1407
Extragonadal	5/20 (25.0)	4/8 (50.0)	1/12 (8.3)	0.0578
Stage IA/B	2/37 (5.4)	0/12 (0.0)	2/25 (8.0)	0.5495
IGCCCG risk group				
Good	17/154 (11.0)	9/37 (24.3)	8/117 (6.8)	0.0061
Intermediate	7/44 (15.9)	2/10 (20.0)	5/34 (14.7)	0.6490
Poor	14/37 (37.8)	5/10 (50.0)	9/27 (33.3)	0.4537

[a]Histological confirmation was not available at the time of initial treatment start date for 1 patient. FN, febrile neutropenia; G-CSF, granulocyte-colony stimulating factor; NSGCT, non-seminomatous germ cell tumor; IGCCCG, International Germ Cell Cancer Collaborative Group.

Table V. Cause of death.

Cause of death	Patients, n (%)	No prophylaxis, n (%)	G-CSF prophylaxis, n (%)	P-value[a]
Death during treatment	11 (2.9)	6 (5.0)	5 (1.9)	0.1071
Disease progression	39 (10.1)	19 (15.7)	20 (7.6)	0.0180
Unknown	1 (0.3)	0 (0.0)	1 (0.4)	NA
Second primary malignancy	1 (0.3)	0 (0.0)	1 (0.4)	NA
Death unrelated to cancer	1 (0.3)	1 (0.8)	0 (0.0)	NA

[a]Death rate in the no prophylaxis group compared with the G-CSF prophylaxis group. G-CSF, granulocyte-colony stimulating factor; NA, not analyzed.

results are shown in Table VI. Patients with NSGCT histology that received G-CSF prophylaxis exhibited a significantly prolonged OS time (HR, 0.52; 95% CI, 0.29-0.93; P=0.0172) compared with those without it. An increased OS rate (HR, 0.56; 95% CI, 0.28-1.09; P=0.0607) was observed in patients that received G-CSF prophylaxis with a gonadal tumor, compared with those without prophylaxis, although the difference was not significant. An increased OS rate (HR=0.28; 95% CI, 0.04-1.92; P=0.1390) occurred in patients taking prophylaxis who were also categorized as good-risk, although the difference was not significant (P=0.1390; Fig. 3A). No statistically significant difference in OS rate was observed in intermediate risk (P=0.8968; Fig. 3B) or poor risk (P=0.6783; Fig. 3C) groups when comparing patients with and without prophylaxis. The results of the multivariate analysis demonstrated that only the IGCCCG risk class was associated with survival, while the remaining results, including those for patients who received G-CSF were not statistically significant (P=0.06; Table VII). Notably, patients who received G-CSF prophylaxis exhibited a reduced death rate due to disease progression (7.6 vs. 15.7%; P=0.0180) compared with patients without prophylaxis (Table V).

No statistically significant difference was observed between the OS of patients with or without G-CSF prophylaxis, following a subgroup analysis of patients receiving a BEP chemotherapy regimen (Table VIII).

Discussion

FN is a complication that often occurs during the course of chemotherapy, leading to prolonged hospital stays, often increasing morbidity and mortality rates (26,27). In the present study, patients receiving primary G-CSF prophylaxis exhibited markedly lower FN incidence rates than patients without it. A decreased FN incidence occurred in patients receiving pegfilgrastim, compared with those receiving filgrastim. These results may suggest that patients have lower compliance to filgrastim than pegfilgrastim. Filgrastim was selected as the main prophylaxis in the inpatient setting for hospitalized patients with a poor performance status. Researchers have previously identified poor performance status as an FN risk factor (12). In the present study, the highest FN incidence was observed in the first chemotherapy cycle, similar to the results of previous studies (12,28). The highest FN incidence in the

Table VI. Overall survival.

Variables	G-CSF prophylaxis, n	No G-CSF prophylaxis, n	HR	Lower 95% CI	Upper 95% CI	P-value
All patients	264	121	0.54	0.31	0.96	0.0235
Stage IA/B	27	12	NA	NA	NA	NA
IGCCCG risk group						
Good	151	55	0.28	0.04	1.92	0.1390
Intermediate	37	14	0.85	0.07	10.11	0.8968
Poor	49	40	0.88	0.48	1.61	0.6783
Chemotherapy regimen						
BEP	204	69	0.63	0.27	1.46	0.2341
EP	32	19	NA	NA	NA	NA
Other	28	33	1.11	0.50	2.49	0.7964
Tumor histology						
Seminoma	65	22	NA	NA	NA	NA
NSGCT	193	99	0.52	0.29	0.93	0.0172
Primary tumor location						
Gonadal	241	103	0.56	0.28	1.09	0.0607
Extragonadal	23	18	0.70	0.24	2.10	0.5178

G-CSF, granulocyte-colony stimulating factor; HR, hazard ratio; 95% CI, 95% confidence interval; IGCCCG, International Germ Cell Cancer Collaborative Group; BEP, bleomycin, etoposide and cisplatin; EP, etoposide and cisplatin; NA, not analyzed; NSGCT, non-seminomatous germ cell tumor.

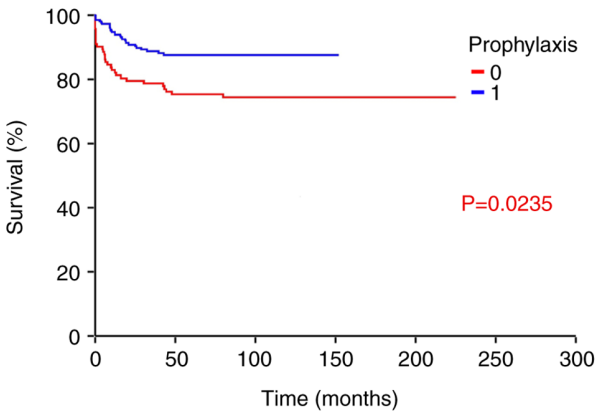

Figure 2. G-CSF prophylaxis and overall survival. Kaplan-Meier analysis determined estimates of probabilities of overall survival, according to primary G-CSF prophylaxis in patients with testicular germ cell tumors (n=385) (hazard ratio, 0.54; 95% confidence interval, 0.31-0.96; P=0.0235). 0, no primary G-CSF prophylaxis; 1, primary G-CSF prophylaxis with filgrastim/pegfilgrastim; G-CSF, granulocyte-colony stimulating factor.

first chemotherapy cycle may also be associated with the use of G-CSF in subsequent cycles, if the patient developed FN in the first cycle, and/or following dose reductions in patients that previously experienced FN. Hematological toxicity is also more pronounced in patients with lower baseline neutrophil and lymphocyte counts, which may be associated with tumor-induced immunosuppression (29,30).

In the present study, an FN incidence rate of 34.7% was observed in patients without G-CSF prophylaxis, which was notably higher than the results reported in previous studies (11,12,31). However, Nishikawa *et al* (9) reported a high incidence rate of 39.5%. In the present study, FN incidence was particularly high in patients treated with T-BEP and VIP. Notably, primary prophylaxis was not mandatory in the phase II trial by Mardiak *et al* (32) using the T-BEP regimen. One factor accounting for the discrepancy may be the difference in study populations. Terbuch *et al* (12) reported a 17% FN incidence rate. However, patients with poor-risk disease accounted for only 12% of all metastatic patients in the previous study, compared with 23.1% observed in the present study. Furthermore, the results of the present study demonstrated that FN incidence was positively associated with IGCCCG risk. While patients with good-risk disease exhibited an FN incidence of 21.8%, the FN incidence was 62.5% in poor-risk disease patients without primary G-CSF prophylaxis. These results are comparable with those of previous studies (16,17), stating that poor-risk disease is a risk factor for FN. Therefore, the study population structure must be considered while comparing FN incidence rates. Furthermore, even patients with good-risk disease exhibited higher levels of FN incidence (21.8%) in the present study, compared with the results of previous studies (12,33). Culine *et al* (33) reported an FN incidence of 7% in patients with good-risk NSGCTs receiving the BEP chemotherapy regimen, and 5% in patients receiving the EP regimen. In addition, Terbuch *et al* (12) revealed an incidence of 17.7% in patients with good-risk disease.

Primary G-CSF prophylaxis reduced FN incidence in patients with NSGCTs and seminomas. However, the

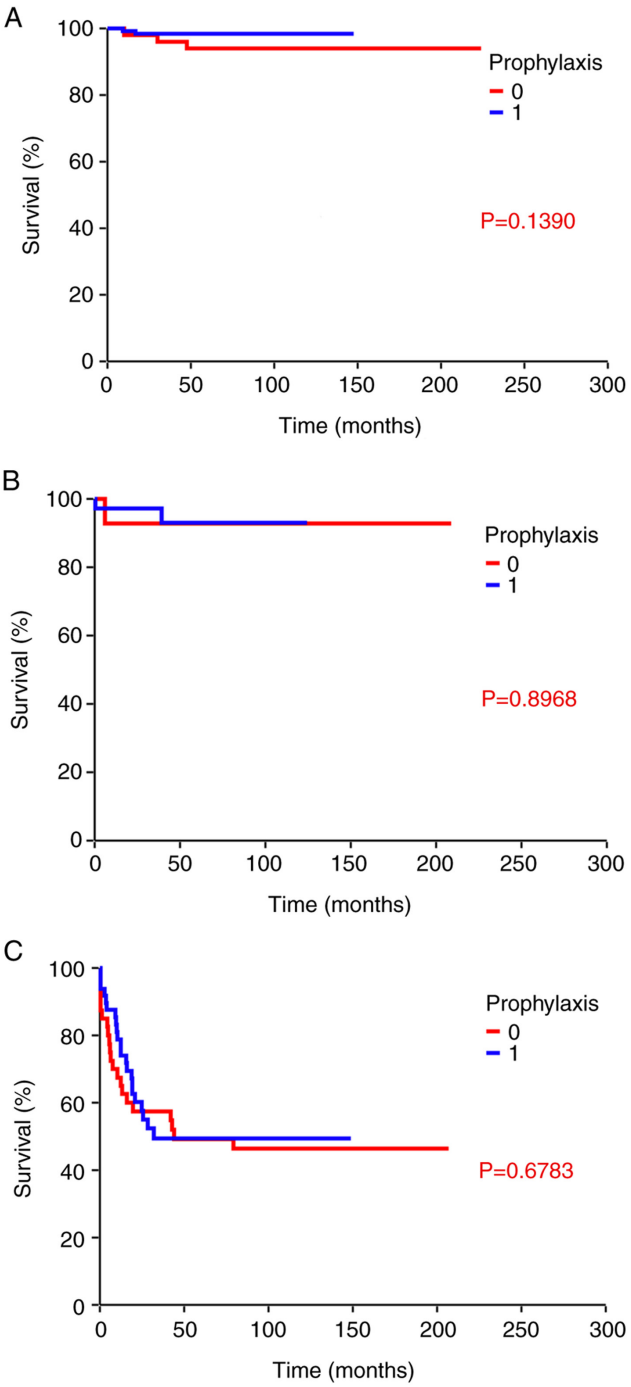

Figure 3. G-CSF prophylaxis and OS by International Germ Cell Cancer Collaboration Group risk group. (A) Kaplan-Meier analysis determined estimates of probabilities of OS, according to primary G-CSF prophylaxis in patients with a good prognosis (n=206) (HR, 0.28; 95% CI, 0.04-1.92; P=0.1390). (B) Kaplan-Meier analysis determined estimates of probabilities of OS, according to primary G-CSF prophylaxis in patients with an intermediate prognosis (n=51) (HR, 0.85; 95% CI, 0.07-10.11; P=0.8968). (C) Kaplan-Meier analysis determined estimates of probabilities of OS, according to primary G-CSF prophylaxis in patients with a poor prognosis (n=89) (HR, 0.88; 95% CI, 0.48-1.61; P=0.6783). 0, no primary G-CSF prophylaxis; 1, primary G-CSF prophylaxis with filgrastim/pegfilgrastim; CI, confidence interval; G-CSF, granulocyte-colony stimulating factor; HR, hazard ratio; OS, overall survival.

difference observed in the seminoma subgroup was not statistically significant. The differences in sample size (seminomas represented only 22.6% of the study population) and lower FN risk in seminomas without prophylaxis may explain these inconclusive results.

Table VII. Multivariate Cox regression analysis of the potential prognostic value of G-CSF.

Variable	HR (95% CI)	P-value
Age (continuous)	1.02 (0.99-1.05)	0.2500
G-CSF (present vs. absent)	0.58 (0.33-1.03)	0.0600
IGCCCG (poor/intermediate vs. good risk)	16.15 (6.37-40.94)	<0.0001

HR, hazard ratio; 95% CI, 95% confidence interval; G-CSF, granulocyte-colony stimulating factor; IGCCCG, International Germ Cell Cancer Collaborative Group.

The chemotherapy regimen influences the FN incidence (32,34,35). Patients treated with T-BEP or VIP exhibit markedly higher hematological toxicity rates than those subjected to the BEP regimen (32,35-37). This may account for the higher incidence of FN in patients with extragonadal GCTs in the present study, as they were treated with VIP or T-BEP regimens more frequently than patients with gonadal tumors. While 16.7% of patients with extragonadal GCT were treated with a VIP chemotherapy regimen, only 2.3% with gonadal tumors received a VIP regimen. T-BEP chemotherapy was also a more frequent treatment choice in patients with extragonadal GCTs (27.7 vs. 21.4%) compared with that in patients with a gonadal primary tumor location. In addition, a higher proportion of patients with extragonadal GCTs had poor-risk disease, compared with those with gonadal GCTs (55.6 vs. 29.1%) (data not shown).

The present data suggested that G-CSF prophylaxis reduced FN incidence in all subgroups, except for patients with stage I disease. The clinical practice guidelines issued in 2010 for the management of FN suggested using an individualized approach, if the expected FN incidence was between 10 and 20% (34). In the present study, two subgroups of patients were in this category: Patients with seminoma and patients receiving EP chemotherapy. Although a numerically lower FN incidence was present in patients receiving primary G-CSF prophylaxis than in patients without prophylaxis, the difference was not statistically significant.

The results of the univariate analysis revealed a statistically significant increase in OS in patients receiving primary G-CSF prophylaxis; however, the results of the multivariate analysis did not verify this finding. Furthermore, when only the subgroup of patients treated with BEP was analyzed, primary G-CSF did not affect OS. Therefore, the observed effect may have been mediated in patients treated with a different regimen than BEP. In addition, cause of death analysis indicated that primary G-CSF reduced deaths during the treatment and was also associated with a lower risk of disease progression. This may be associated with dose modification of chemotherapy and treatment delays, due to FN occurrence in patients without primary prophylaxis. However, other immune-related mechanisms may also contribute to this observation. Previous studies have demonstrated that

Table VIII. Overall survival in patients receiving the BEP regimen.

Variables	G-CSF prophylaxis, n	No G-CSF prophylaxis, n	HR	Lower 95% CI	Upper 95% CI	P-value
Patients receiving BEP	204	69	0.63	0.27	1.46	0.2341
Stage IA/B	26	12	NA	NA	NA	NA
IGCCCG risk group						
Good	117	37	0.25	0.04	1.79	0.1011
Intermediate	34	10	0.66	0.05	9.07	0.7334
Poor	27	10	0.69	0.24	2.00	0.4609
Tumor histology						
Seminoma	31	3	NA	NA	NA	NA
NSGCT	171	66	0.56	0.23	1.36	0.1573
Primary tumor location						
Gonadal	191	61	0.66	0.26	1.67	0.3321
Extragonadal	12	8	0.71	0.10	5.17	0.7269

G-CSF, granulocyte-colony stimulating factor; HR, hazard ratio; 95% CI, 95% confidence interval; IGCCCG, International Germ Cell Cancer Collaborative Group; BEP, bleomycin, etoposide and cisplatin; NA, not analyzed.

immune-related factors, such as programmed death-ligand 1 expression, systemic inflammatory index and serum cytokines, are associated with GCT prognosis (38-40). Therefore, administration of G-CSF may have a pleiotropic effect on the immune system beyond neutrophil count. Consequently, an increased OS rate in patients receiving G-CSF prophylaxis may also be explained by this mechanism (41). Notably, G-CSF mediates the differentiation of granulocyte progenitors into mature granulocytes, including neutrophils, eosinophils and basophils (42,43).

The pro-tumor role of G-CSF has been described in a preclinical study (44). While no detrimental effects have been observed in patients receiving prophylactic G-CSF to prevent chemotherapy-induced neutropenia, further research is required to understand the role of G-CSF in tumor growth, progression, metastasis and treatment outcomes (45).

To the best of our knowledge, the present study is the most expansive study evaluating the effects of primary G-CSF prophylaxis in patients with GCT, reflecting routine clinical practice in a tertiary cancer center. The present study has limitations due to its retrospective, non-randomized and single-center design. The majority of patients received BEP or EP chemotherapy. The number of patients receiving other chemotherapy regimens was not high and was not uniformly distributed among subgroups. Therefore, the results predominantly apply to patients with GCT treated with BEP or EP regimens. The number of patients in several subgroups was also limited. Implementation of primary G-CSF prophylaxis into practice was progressive and there were some patients during the transition period (2006-2007) that did not receive it. It cannot be excluded that during this period the decision for administration of G-CSF prophylaxis was also driven by the perception of the physician of the risk of FN and this could be a potential source of bias; however, from 2008, almost all patients received prophylaxis. In addition, patients that did not receive primary G-CSF prophylaxis received G-CSF prophylaxis in subsequent cycles, secondary to the neutropenia or FN in the previous cycle.

In conclusion, the present retrospective study demonstrated the prophylactic effects of G-CSF on FN incidence in patients with GCT who were treated with first-line chemotherapy for metastatic disease. This effect was most pronounced in more aggressive chemotherapy regimens other than EP or BEP (such as T-BEP and VIP). According to the results of the present study, G-CSF prophylaxis should be considered in daily clinical practice for patients with metastatic GCTs that have been subjected to first-line chemotherapy, particularly in the first cycle in patients with high-risk features.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JM and MM were responsible for conceptualization. NH, MC, KR, KK, JO, PP, VDA, DS, ZSM, JM and MM were responsible for data collection. NH and MM were responsible for the formal analysis. NH, JM and MM were responsible for the methodology. NH and MM were responsible for visualization. NH and MM confirm the authenticity of all the raw data. MM and NH were responsible for writing the original draft, and NH, MC, KR, KK, JO, PP, VDA, DS, ZSM, JM and MM were responsible for writing, reviewing and editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Review Board of the National Cancer Institute (Bratislava, Slovakia) approved the present study, and granted a waiver of consent (approval no. IZLO1) for the collection, analysis and publication of the retrospectively obtained and anonymized data for this non-interventional study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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