The Status of Spermatogenesis in Germ Cell Tumor Bearing Testis and Its Association with Metastatic Disease

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Abstract

We retrospectively reviewed patients with TGCTs that undergone radical orchiectomy and all non-tumoral areas of the orchiectomy specimens were examined for the status of spermatogenesis. Our study demonstrated that only 22.5% of patients with TGCTs had normal spermatogenesis in tumor bearing testis. Impaired spermatogenesis (hypospermatogenesis or no mature spermatozoa) and predominant embryonal carcinoma are associated with advanced stage NSGCT.

Introduction: We aimed to evaluate the status of spermatogenesis detected by histological examination of non-tumoral testicular tissues in tumor bearing testis and its association with advanced stage disease. **Patients and Methods:** We retrospectively reviewed patients with testicular germ cell tumors (TGCTs) that undergone radical orchiectomy. All non-tumoral areas of the orchiectomy specimens were examined for the status of spermatogenesis. Patients were divided into two groups as localized (stage I) and metastatic (stage II-III) disease and analyzed separately for seminomatous (SGCT) and nonseminomatous germ cell tumors (NSGCT). **Results:** Four hundred fifty-four patients were included in our final analysis. Of those, 195 patients had SGCT, and 259 patients had NSGCT. Three hundred and six patients had localized disease at the time of diagnosis. Median (Q1-Q3) age was 31 (26 – 38) years and 102 (22.5%) patients had normal spermatogenesis, 177 (39.0%) patients had hypospermatogenesis and 175 (38.5%) patients had no mature spermatozoa. On multivariate logistic regression analysis, embryonal carcinoma >50% (1.944, 95 %CI 1.054-3.585, $P = .033$) and spermatogenesis status (2.796 95% CI 1.251-6.250, $P = .012$ for hypospermatogenesis, and 3.907, 95% CI 1.692-9.021, $P = .001$ for absence of mature spermatozoa) were independently associated with metastatic NSGCT. However, there was not any variables significantly associated with metastatic SGCT on multivariate logistic regression analysis. **Conclusion:** Our study demonstrated that only 22.5% of patients with TGCTs had normal spermatogenesis in

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tumor bearing testis. Impaired spermatogenesis (hypospermatogenesis or no mature spermatozoa) and predominant embryonal carcinoma are associated with advanced stage NSGCT.

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Introduction

Despite having a low overall incidence, testicular cancer is the most prevalent solid cancer among young men in western nations.[1] Its prevalence has been rising over the past few decades, especially in industrialized countries.[2] In 90%-95% of cases, testicular germ cell tumors (TGCTs) are the most common histology and classified into 2 groups: seminomatous germ cell tumors (SGCT) and non-seminomatous germ cell tumors (NSGCT)[3]. SGCT and NSGCT are commonly detected at fourth and third decade of life, respectively.[3]

Testicular cancer has good prognosis with 0.22/100000 person-years global age-standardized rate of mortality.[1] At the time of diagnosis, 75%-80% of the patients with SGCT and 55%-64% of the patients with NSGCT have stage I disease.[4,5] While these patients can be cured with just radical orchiectomy, additional adjuvant therapies such as chemotherapy, radiotherapy and in some patients, post-chemotherapy retroperitoneal lymph node dissection are required in metastatic patients. With the introduction of cisplatin-based chemotherapies, even metastatic TGCTs can be cured at a high rate. In these circumstances, accurate staging and evaluating the prognostic factors become very important in the choice of treatment.

Impaired fertility and testicular dysgenesis syndrome (which includes undescended testis) are well-known risk factors for the development of TGCTs.[3,6] A previous study demonstrated that more than 50% of the patients with TGCTs had reduced sperm concentration before radical orchiectomy.[7] We hypothesized that, since infertility is a well-known factor in the development of testicular cancer, there may be an association between impaired spermatogenesis and cancer aggressiveness. However, studies about the relationship between subfertility and tumor aggressiveness is very limited.

In this study, we aimed to evaluate the status of spermatogenesis detected by histological examination of non-tumoral testicular tissue in tumor bearing testis and its association with advanced stage disease.

Patients and Methods

We conducted this retrospective study after obtaining institutional review board (IRB) approval (IRB No: 2021/0676, decision date: December 22, 2021). We retrospectively reviewed patients with suspicion of testicular cancer that underwent radical orchiectomy between January 2009 and December 2021 in 5 different tertiary referral centers.

Patients older than 17 years of age and whose radical orchiectomy specimens could be found through archive scanning were included in our study. Patients with non-TGCTs pathology, bilateral synchronic tumors, incomplete clinical data and without non-tumoral testicular parenchyma were excluded from the study. None of the patients had medication due to infertility or history of undescended testis.

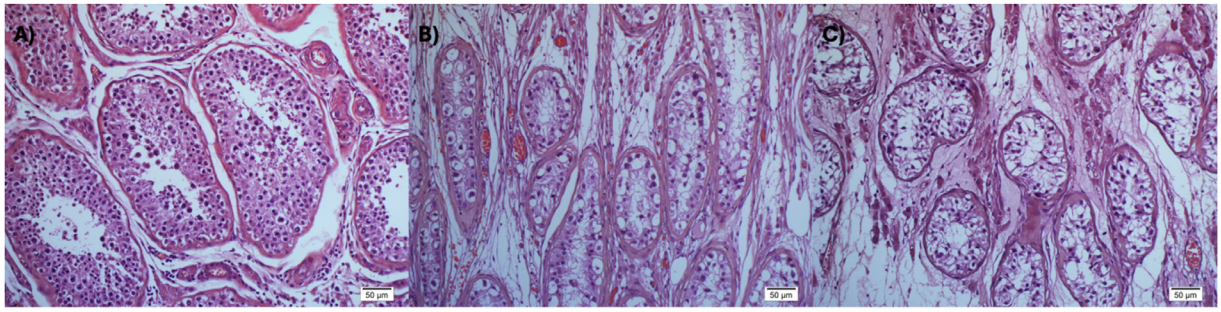
Patients' age at the time of radical orchiectomy, tumor laterality, pre-operative serum levels of b-human chorionic gonadotropin (b-hCG), a-fetoprotein (AFP) and lactate dehydrogenase (LDH), tumor histology, maximum tumor diameter, presence of lymphovascular invasion (LVI) and rete testis invasion, pathological T stage, presence of lymph nodes or metastasis were recorded. Clinical staging was performed with contrast enhanced computerized tomography or magnetic resonance imaging. After radical orchiectomy and clinical staging, patients were treated or followed with physical examination, tumor markers and contrast enhanced computerized tomography or magnetic resonance imaging according to relevant guidelines.

All archived slides of pathological specimens were re-evaluated by dedicated pathologists experienced in uro-oncology, andrology and blinded to the clinical data. All non-tumoral areas, especially areas far from the tumor, were examined for the status of spermatogenesis and spermatogenesis was classified into 3 groups: normal spermatogenesis, hypospermatogenesis, and absence of mature spermatozoa (germ cell arrest, Sertoli cell-only syndrome or seminiferous tubule hyalinization) according to classification published by McLachlan et al. (Figure 1).[8] If the specimen had areas of all classifications, we accepted the classification with better maturation as the final classification. Spermatogenesis status and relevant tumor characteristics were re-evaluated by 5 different uro-pathologists at the respective centers where orchiectomy was performed. Anatomical extent of the diseases was determined according to the 2016 Tumor, Node, Metastasis (TNM) classification of the International Union Against Cancer.[9] Patients were divided into two groups as localized (stage I) and advanced TGCTs (stage II-III) and evaluated and analyzed separately for SGCT and NSGCT.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 26. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. Patients' clinical and pathological variables were reported with descriptive statistics. The proportions of patients with metastasis were presented by laterality, embryonal carcinoma <50%, presence of LVI, presence of rete testis invasion, spermatogenesis status and T stage using cross tabulations. Descriptive analyses were presented using medians and interquartile range (IQR) for the non-normally distributed variables. The univariate analyses to identify variables associated with advanced TGCTs was investigated using Chi-square in analysis of laterality, embryonal

Figure 1 Histologic evaluation of spermatogenesis (haematoxylin and eosinX20); (A) Normal spermatogenesis (B) hypospermatogenesis (C) Absence of mature spermatozoa (sertoli cell only syndrome).



carcinoma <50%, presence of LVI, presence of rete testis invasion, spermatogenesis status and T stage and Mann-Whitney U tests in analysis of age, preoperative serum levels of AFP, β-hCG, LDH and tumor size. For the multivariate analyses, the possible factors identified with univariate analyses (P value < .2) were further entered into the logistic regression analysis with enter method to determine the factors that may be independently related to advanced TGCTs. Hosmer-Lemeshow goodness of fit statistics were used to assess model fit. A 5% type-I error level was used to infer statistical significance.

Results

From 521 patients reviewed for the study, after exclusion 454 were valid for final analysis (Figure 2). Of them, 195 patients had SGCT, and 259 patients had NSGCT. While 306 (67.40%) patients had localized TGCTs at the time of diagnosis, 148 (32.60%) patients had advanced TGCTs. Median (IQR) age was 31 (26-38) years. Of the patients, 102 (22.5%) had normal spermatogenesis, 177 (39.0%) had hypospermatogenesis and 175 (38.5%) had no mature spermatozoa.

In the NSGCT group, 105 patients (40.5%) presented with advanced and 154 (59.5%) with localized disease. There were no statistically significant differences in median age (30 vs 28 years, P = .127), tumor laterality (P = .565), and median tumor size (40 vs 42 mm, P = .326) between localized and advanced NSGCT (Table 1). Patients with advanced NSGCT had significantly higher preoperative levels of AFP (24.9 vs 84.4 ng/ml, P = .014), b-hCG (3.7 vs 27 IU/L, P < .001), LDH (217 vs 278 IU/L, P < .001) and higher rates of predominant embryonal carcinoma component >50% (34.4% vs 46.6%, P = .024) and LVI (49.9% vs 63.8, P < .001). Finally, there was statistically significant differences between the two groups according to spermatogenesis status (P < .001). A higher rate of advanced diseases was observed in patients with impaired spermatogenesis. Normal spermatogenesis was detected 30.51% and 11.42% for patients with localized and advanced NSGCT, respectively (Table 1).

In the SGCT group, 43 (22.05%) patients were presented with advanced and 152 (77.94%) with localized disease. The median (IQR) age was 34 (29-42) and 41 (31-43) years for localized and advanced patients respectively (P = .87). There was no statistically significant different between the two groups according to preoperative serum levels of AFP (2.6 vs 2.4 ng/ml, P = .594), however patients with advanced SGCT had higher levels of preoperative serum levels of b-hCG (1.8 vs 2.4 IU/L, P = .010) and LDH (220.0 vs 345.5 IU/L, P < .001). In addition to this, patients with advanced SGCT had larger tumor size (45.0 vs 60.0 mm, P = .002) than patients with localized SGCT. Last, there was no statistically significant difference between the two groups according to presence of LVI (28.3% vs 30.2%, P = 804), rete testis invasion (34.9% vs 46.5%, 0.164) and spermatogenesis status (P = .118) (Table 1).

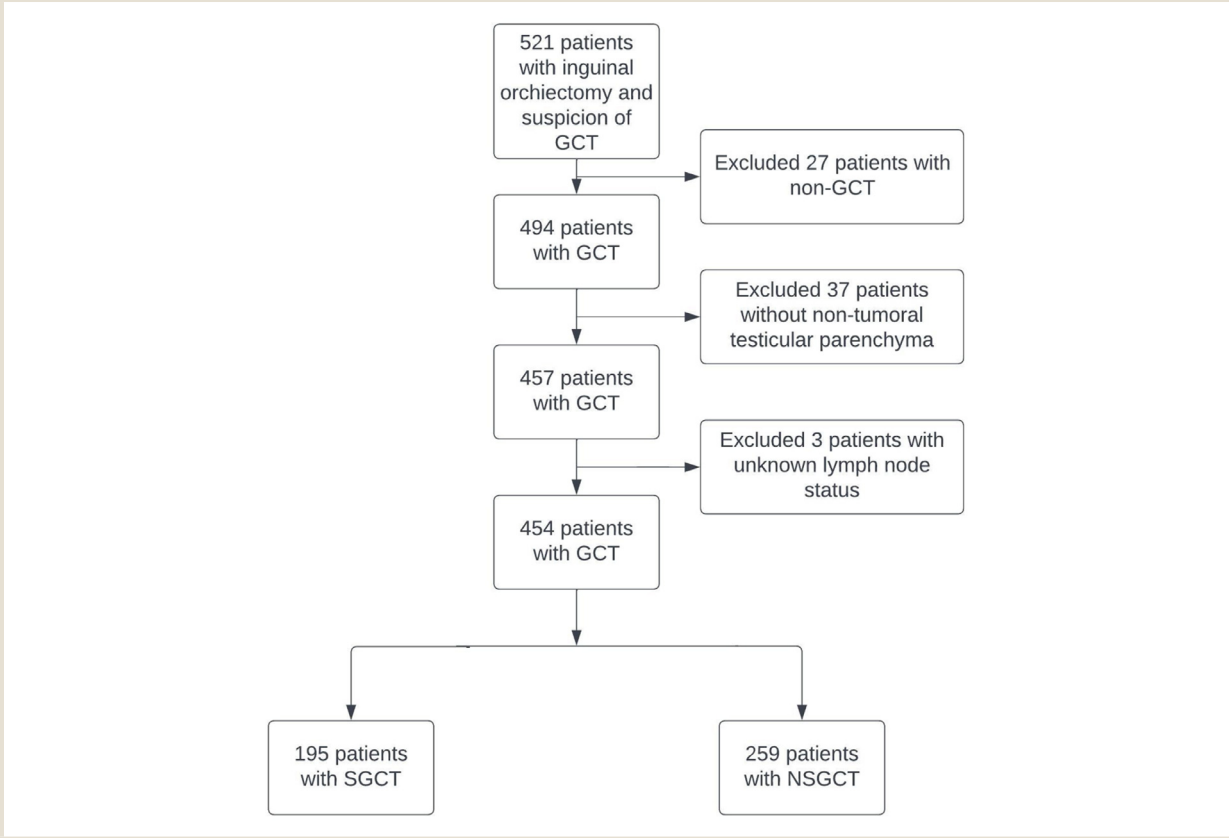
On multivariate logistic regression analysis, embryonal carcinoma >50% (1.944, 95% CI 1.054-3.585, P = .033), hypospermatogenesis (2.796, 95% CI 1.251-6.250, P = .012) and absence of mature spermatozoa (3.907, 95% CI 1.692-9.021, P = .001) were significantly associated with advanced stage NSGCT. However, there was no variables significantly associated with advanced stage SGCT on multivariate logistic regression analysis (Table 2).

Recurrence was detected in 10 of 154 patients with localized NSGCT at a median (IQR) 25 (10-50) months of follow-up. Among those patients, 2 had normal spermatogenesis, 5 had hypospermatogenesis and 3 had no mature spermatozoa in their orchiectomy specimens. In the group of patients with SGCT, recurrence was detected in 9 of 152 localized patients during median (IQR) 26 (12-52) month follow-up. Among those patients, 1 had normal spermatogenesis, 1 had hypospermatogenesis and 7 had no mature spermatozoa in their orchiectomy specimens.

Discussion

It is already known that testicular dysgenesis syndrome includes undescended testis, hypospadias or infertility is an important risk factor for testicular cancer.[3] Infertile men have higher prevalence of germ cell neoplasia in situ (GCNIS), the precursor lesion of GCN, than general male population.[8,10] Histological analysis of testicular tissues of patients with GCNIS, showed a decrease in the diameters of the seminiferous tubules, thinning of the basement membrane, and impaired spermatogenesis.[11] In addition, similar risk factors such as genetic defects (sex chromosome aneuploidy, gene deletions/mutations) and environmental factors (such as

Figure 2 Flowchart for excluded and included number of patients. GCT: germ cell tumor; NSGCT: non-seminomatous germ cell tumor; SGCT: seminomatous germ cell tumor.



endocrine disrupters) for testicular dysgenesis syndrome and testicular cancer suggest a strong relationship between these two.[12] GCNIS, which is a precursor cell of TGCTs, originates from arrested fetal germ cells; thus, TGCTs is a developmental disease of germ-cell differentiation. Some studies of gene expression at the protein and RNA level revealed the close similarity of GCNIS to primitive germ cells and gonocytes. Thus, the crucial initial event is the arrest of germ-cell differentiation, which may be followed by malignant transformation and overt germ-cell cancer in early adulthood, typically following puberty. This hypothesis may explain the biological mechanism of the relationship between impaired spermatogenesis and testicular cancers.[13]

In this study, we demonstrated that 77.5% of patients with testicular cancer had various degrees of impaired spermatogenesis (39.0% hypospermatogenesis and 38.5% no mature spermatozoa) in ipsilateral testis. To date, several studies have investigated the status of spermatogenesis in patients with testicular cancer. In 2018, Moody et al. conducted a study to investigate status of spermatogenesis in tumor bearing testis in patients with testicular cancer and reviewed 103 TGCTs. They reported that 70% of patients had spermatogenesis and in 38% of these patients, spermatogenesis was focal.[14] There are several hypotheses about the mechanism of reduced spermatogenesis in patients with TGCTs. This deterioration may be due to substances produced by the tumor such as b-hCG and AFP or due to tumor mediated cytokines such as tumor necrosis factor and interleukin 1. b-hCG may affect Leydig cell function and consequently local spermatogenesis because it functions as a luteinizing hormone analogue.[15] In another study, Hayashi et al. investigated the association between spermatogenesis and serum b-hCG levels and concluded that there was a significant linear relationship ($r = -0.82$; $P < .005$) between the serum b-hCG levels and the mean Johnsen's score count in contralateral testicular tissues.[16] Some other reports claimed that local tumor suppressive effect of large tumors may cause impaired spermatogenesis.[17-19] It is demonstrated that spermatogenesis was more impaired in the vicinity of the tumor than in distant areas.[15] Based on this information, we made our examination to cover all non-tumoral tissue, especially in areas far from the tumor. Similar to this study, we also observed that spermatogenesis was more impaired in the areas close to the tumor. In a study that investigated the predictor factors of spermatogenesis in orchiectomy specimens, authors stated that tumor size was the only factor associated with spermatogenesis status. However, authors also stated that spermatozoa could be identified even in the presence of very large tumors.[19] Similar to this study, in our study, we could also find a mature spermatozoa even in some tumors bigger than 10cm.

Although the relationship between impaired spermatogenesis and testicular cancer development has been known for many years, there are very few studies examining the relationship between impaired

Table 1 Clinicopathologic Characteristics of the Patients With Testicular Cancer and Underwent Radical Orchiectomy Between January 2009 and December 2021

Parameter	Localized NSGCT ($n = 154$)	Advanced NSGCT ($n = 105$)	*P* Value	Localized SGCT ($n = 152$)	Advanced SGCT ($n = 43$)	*P* Value
Age (years), median (Q1-Q3)	30 (24-35)	28 (23-33)	.127[a]	34 (29-42)	41 (31-43)	.087[a]
Laterality, *n* (%)			.565[b]			.389[b]
Right	78 (50.64)	57 (54.28)		82 (53.94)	20 (46.51)	
Left	76 (49.36)	48 (45.72)		70 (46.06)	23 (53.49)	
Preoperative serum levels of AFP (ng/ml), median (Q1-Q3)	24.9 (4.0-150.2)	84.4 (7.8-600.0)	.014[a]	2.6 (1.9-3.5)	2.4 (2.0-4.2)	.594[a]
Preoperative serum levels of β-hCG (IU/L), median (Q1-Q3)	3.7 (1.2-69.0)	27 (2.7-855.0)	< .001[a]	1.8 (0.2-4.5)	2.4 (0.9-36.0)	.010[a]
Preoperative serum levels of LDH (IU/L), median (Q1-Q3)	217.0 (177.0-298.0)	278.0 (218.0-455.0)	< .001[a]	220.0 (172.5-298.0)	345.5 (209.0-542.2)	< .001[a]
Tumor size (mm), median (Q1-Q3)	40.0 (28.0-55.0)	42.0 (26.0-55.0)	.326[a]	45.0 (30.0-60.0)	60.0 (40.0-80.0)	.002[a]
Embryonal carcinoma >50%, *n* (%)			.024[b]			
No	88 (57.14)	45 (42.85)				
Yes	66 (42.86)	60 (57.15)				
Presence of LVI, *n* (%)			< .001[b]			.804[b]
No	91 (59.09)	38 (36.19)		109 (71.71)	30 (69.76)	
Yes	63 (40.91)	67 (63.81)		43 (28.29)	13 (30.24)	
Presence of rete testis invasion, *n* (%)			.048[b]			.164[b]
No	101 (65.58)	56 (53.33)		99 (65.13)	23 (53.48)	
Yes	53 (34.41)	49 (46.67)		53 (34.87)	20 (46.52)	
Spermatogenesis status, *n* (%)			< .001[b]			.118[b]
Normal	47 (30.51)	12 (11.42)		38 (25.00)	5 (11.62)	
Hypospermatogenesis	63 (40.90)	45 (42.85)		54 (35.52)	15 (34.88)	
Absence of mature spermatozoa	44 (28.59)	48 (45.73)		60 (44.48)	23 (53.50)	
T stage, *n* (%)			.001[b]			.819[b]
pT1	84 (54.54)	33 (31.42)		99 (65.13)	26 (60.46)	
pT2	64 (41.55)	59 (56.19)		48 (31.57)	15 (34.88)	
pT3	6 (3.91)	12 (12.39)		5 (3.30)	2 (4.66)	
pT4	0 (0.0)	1 (100.0)				

[a] Mann-Whitney U Test.
[b] Pearson chi-square.AFP: α-fetoprotein; LDH: lactate dehydrogenase; LVI: lymphovascular invasion; NSGCT: Non-seminomatous germ cell tumors; β-hCG: β-human chorionic gonadotropin.

spermatogenesis and testicular cancer aggressiveness. To date, in our knowledge only one study investigated this issue and the authors reviewed pathology specimens of 267 patients with TGCTs. In this cohort, 115 (43%) patients had NSGCT, and 152 (57%) patients had SGCT. As a result, they stated that spermatogenesis was identified 80.7% of patients with NSGCT and 71.5% of patients with SGCT. In multivariate analysis, lack of spermatogenesis in tumor bearing testis was associated with advanced stage diseases in patients with NSGCT, but not in patients with SGCT.[20] In our study, we divided patients into 3 groups in terms of spermatogenesis status. Unlike the aforementioned, our data strongly suggest that not only the absence of spermatogenesis, but also the presence of hypospermatogenesis is associated with advance stage NSGCT. Similarly to the study published by Halstuch et al., we also demonstrated that there was no association between spermatogenesis status and tumor aggressiveness in patients with SGCT.[20] It is valuable that we confirmed this finding with more balanced and larger cohort which is important for proper statistical analysis (152 stage I, 43 stage II-III, a total of 195 patients with SGCT).

Approximately 15% of patients with localized SGCT and 30% of patients with localized NSGCT have subclinical metastatic cancer and will relapse if they do not receive adjuvant treatments.[3] In these circumstances, it is very important to be able to identify predictive factors for recurrence in order to select patients who need adjuvant therapy in clinical stage I disease. Tumor size and invasion of the rete testis for SGCT and presence of embryonal carcinoma

Table 2 Multivariate Logistic Regression Analysis for Predicting Advance Stage Testicular Cancer

	SGCT		NSGCT	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age	1.026 (0.992-1.062)	.135	0.965 (0.932-1.000)	.052
Preoperative serum levels of AFP	-	-	1.000 (1.000-1.000)	.081
Preoperative serum levels of β-hCG	1.000 (0.999-1.002)	.457	1.000 (1.000-1.000)	.240
Preoperative serum levels of LDH	1.000 (1.000-1.001)	.630	1.001 (0.999-1.002)	.243
Tumor size	2.040 (0.644-6.460)	.225	-	-
Embryonal carcinoma >50%	-	-	1.944 (1.054-3.585)	.033
Presence of LVI	-	-	1.178 (0.294-4.723)	.817
Presence of rete testis invasion	1.762 (0.846-3.669)	.130	1.302 (0.691-2.452)	.414
Spermatogenesis status		.238		.006
Normal	Reference		Reference	
Hypospermatogenesis	1.497 (0.469-4.872)	.503	2.796 (1.251-6.250)	.012
Absence of mature spermatozoa	2.354 (0.788-7.035)	.125	3.907 (1.692-9.021)	.001
Tumor Stage	-	-		.822
pT1			Reference	
pT2			1.263 (0.306-5.211)	.746
pT3			1.786 (0.275-11.612)	.544

AFP: α-fetoprotein; LDH: lactate dehydrogenase; LVI: lymphovascular invasion; NSGCT: Non-seminomatous germ cell tumors; SGCT: Seminomatous germ cell tumors; β-hCG: β-human chorionic gonadotropin.

and LVI in peri-tumoral tissue for NSGCT are well-known risk factors for occult metastatic disease in stage I GCT.[21,22] Embryonal carcinoma has totipotential capacity and can differentiate to other NSGCT.[23] In previous studies, the presence of embryonal carcinoma has been associated with occult metastatic diseases in clinical stage I diseases and have similar ORs with embryonal carcinoma >50%.[21] In our study presence of predominant embryonal carcinoma and impaired spermatogenesis were associated with advanced stage NSGCT, however we could not investigate the predictive ability for recurrence of these factors in patients with clinical stage I disease. We had recurrence in 10 (6.49%) patients with NSGCT and 9 (5.92%) patients with SGCT. In our study the low recurrence rates (10 (6.49%) patients with NSGCT and 9 (5.92%) patients with SGCT) precluded any sound investigation of the predictive value of these factors in clinical stage I disease. However, our observations are hypothesis generating and should encourage studies wither larger cohorts and longer follow-up that investigate the potential predictive value of spermatogenesis status on recurrence in patients with stage I TGCTs.

In recent years, a significant number of studies on testicular cancer have been carried out for biomarkers and minimal invasive treatments. Over the past ten years, research has shown that microRNAs are more effective than traditional serum tumor markers for original tumor diagnosis, follow-up surveillance, and recurrence prediction. The most reliable of these is miR-371a3p with more than 90% sensitivity and specificity.[24] These predictive factors can enable the diagnosis of recurrent tumors in the early period and thus the treatments with less morbidity as possible, such as unilateral robot assisted retroperitoneal lymph node dissection.[25]

Although our study has strengths such as multicenter design and larger size than other studies investigating the relationship between spermatogenesis and testicular cancer, it also has some limitations. The retrospective character of our data leads to a potential bias and the selection of patients from tertiary referral centers might limit the generalizability of the findings. Besides this, pathological specimens were not re-evaluated by a single pathologist. However, all the pathologists involved are experienced in the field of uropathology and had a good inter-communication in case of undetermined or doubtful pathology findings. Last, the lack of systematic preoperative semen analysis in our cohort, precluded investigation of the relationship between preoperative sperm count and tumor aggressiveness.

Conclusion

Our study demonstrated that only 22.5% of patients with TGCT had normal spermatogenesis in tumor bearing testis. İmpaired spermatogenesis (hypospermatogenesis or no-mature spermatozoa) and predominant embryonal carcinoma are associated with advanced stage NSGCT. Studies with a larger number of patients and longer follow-up are needed to determine the potential value of impaired spermatogenesis in terms of predicting survival or recurrence. Also, our study points towards the need for prospective studies with systematic preoperative semen analysis to further validate the findings and explore the relationship between preoperative sperm count and tumor aggressiveness.

Clinical Practice Points

- Impaired fertility and testicular dysgenesis syndrome (which includes undescended testis) are well-known risk factors for the development of TGCTs. A previous study demonstrated that more than 50% of the patients with TGCTs had reduced sperm concentration before radical orchiectomy.

- We hypothesized that, since infertility is a well-known factor in the development of testicular cancer, there may be an association between impaired spermatogenesis and cancer aggressiveness.
- However, studies about the relationship between subfertility and tumor aggressiveness is very limited.
- Our study demonstrated that most patients with testicular cancer had impaired spermatogenesis in tumor bearing testis and the severity of impairment in spermatogenesis correlated with the disease aggressiveness in NSGCTs. In addition, further studies may reveal whether impaired spermatogenesis is a predictive factor for recurrence in stage 1 patients.

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Disclosure

The authors have stated that they have no conflicts of interest.

CRediT authorship contribution statement

Meftun Culpan: Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Asif Yildirim:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Data curation. **Sidika Seyma Ozkanli:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Data curation. **Resul Sobay:** Writing – review & editing, Resources, Investigation, Data curation. **Fatıma Gursoy:** Writing – review & editing, Resources, Investigation, Data curation. **Ramazan Topaktas:** Writing – review & editing, Resources, Investigation, Data curation. **Gulistan Gumrukcu:** Writing – review & editing, Resources, Investigation, Data curation. **Sacit Nuri Gorgel:** Writing – review & editing, Resources, Investigation, Data curation. **Fulya Cakalagaoglu:** Writing – review & editing, Resources, Investigation, Data curation. **Gokhan Cil:** Writing – review & editing, Resources, Investigation, Data curation. **Şule Ozsoy:** Writing – review & editing, Resources, Investigation, Data curation. **Humeyra Gunel:** Writing – review & editing, Resources, Investigation, Data curation. **Eyup Veli Kucuk:** Writing – review & editing, Resources, Investigation, Data curation. **Metin Ishak Ozturk:** Writing – review & editing, Resources, Investigation, Data curation. **Yigit Akin:** Writing – review & editing, Resources, Investigation, Data curation. **Ahmet Yaser Muslumanoglu:** Writing – review & editing, Resources, Investigation, Data curation. **Abdullah Aydin:** Writing – review & editing, Resources, Investigation, Data curation. **Maria Del Pilar Laguna Pes:** Writing – review & editing, Supervision, Resources, Investigation, Data curation.

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