Prevalence of Intratubular Germ Cell Neoplasia and Multifocality in Testicular Germ Cell Tumors ≤ 2 cm: Relationship With Other Pathological Features

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Abstract

We aimed to determine the prevalence of testicular intraepithelial neoplasia (TIN) and multifocality in men potentially eligible for testis-sparing surgery. The prevalence of multifocality and TIN was decreased in smaller tumors (≤ 1 cm) and increased when the index mass tumor diameter was ≥ 1.1 cm. We suggest that this be given particular attention during testis-sparing surgery.

Introduction: The aim of this study was to determine the prevalence of TIN and multifocality in men undergoing radical orchiectomy for testicular germ cell tumor (TGCT), and among those with a main tumor size ≤ 2 cm, potentially eligible for testis-sparing surgery. **Patients and Methods:** Orchiectomy specimens from 126 consecutive patients treated for TGCT tumor between 2003 and 2012 were included. Multifocality was defined as a distinct tumor focus with a diameter ≥ 1 mm separable from the main tumor mass. Uni- and multivariate logistic regression was performed to identify the association between pathological variables and multifocality and to identify variables for predicting clinical stage II to III and pathological stage ≥ pT2. **Results:** Of the 126 patients, 103 (82.0%) had clinical stage I cancer at presentation and 23 (18.0%) had clinical stage II to III. The median size of the primary tumor mass was 3.7 cm (range, 0.5-12 cm) in multifocality and 3.0 cm (range, 0.6-8.0 cm) in monofocality, respectively ($P < .05$). The prevalence of multifocality and TIN was lower in the presence of a smaller main tumor mass (≤ 1 cm) compared with tumors 1.1 to 2.0 cm ($P < .05$), and increased when the index mass tumor diameter was ≥ 2 cm (P trend $< .05$). No association was found between tumor histology and multifocality ($P = .95$) or TIN ($P = .54$) using the χ^2 test. **Conclusion:** The prevalence of multifocality and TIN was decreased in smaller tumors (≤ 1 cm) and increased when the index mass tumor diameter was ≥ 1.1 cm.

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Introduction

Testicular cancer represents between 1% and 1.5% of male neoplasms and 5% of urological tumors in general, with 3 to 10 new cases occurring per 100,000 men per year in Western society.[1-3] The peak incidence is in the third and fourth decade of life and there is a clear trend toward an increased testicular cancer incidence in the past 30 years in most of the industrialized countries especially for seminoma.[4] The histological type varies, although there is a clear predominance (90%-95%) of testicular germ cell tumors (TGCTs).[1,5,6] Standard therapy for testicular cancer is radical orchiectomy. However, in men with bilateral tumors or tumor in a solitary testis, removing the gonads invariably results in infertility and testosterone deficiency. To avoid these consequences, in very selected patients, an organ-preserving surgery can be performed when the tumor volume is < 30% of the testicular volume

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and surgical rules are respected.[7] In those cases, the rate of associated testicular intraepithelial neoplasia (TIN), a preinvasive stage of TGCT, is high (at least up to 82%), and all patients must be treated with adjuvant radiotherapy (16-20 Gy) at some point.[7] In fact, TIN is frequently observed in the testicular parenchyma adjacent to invasive cancer and studies show that approximately 50% of men with TIN are at risk for invasive tumors within 5 years.[8] Furthermore, multifocality could be a finding in TGCT cases and in those with seminomatous histology, as reported in a recent study. The significance of this finding is not well understood.[9,10] For these reasons, concerns have been raised with respect to the long-term oncological safety of organ-sparing surgery. The aim of our study was to determine the prevalence of TIN and multifocality in men undergoing radical orchiectomy for TGCT. Furthermore, we aimed to evaluate the prevalence of multifocality and TIN among testicular cancer with a main tumor size $\leq$ 2 cm, potentially eligible for testis-sparing surgery.

Patients and Methods

Orchiectomy specimens from 126 consecutive patients treated for TGCT tumor between 2003 and 2012 were included in the study. The study was approved by our institutional research ethics committee and informed consent was obtained from all of the subjects.

All patients underwent physical examination, assessment of serum tumor markers, scrotal ultrasound, and abdominal and chest computed tomography scans. Staging was done using the tumor, node, metastases classification of malignant tumors of 2002.

Clinical stage was assigned as: stage I, tumor confined to the testis; stage II, regional retroperitoneal node metastasis; and stage III, distant metastasis beyond the regional retroperitoneal nodes. Risk stratification of patients with metastatic disease at presentation was performed according to International Germ Cell Cancer Collaborative Group criteria.[7] All orchiectomy specimens were processed and analyzed by a single dedicated uropathologist and TGCTs were classified according to the World Health Organization classification. For each sample of orchiectomy specimen, multiple tissue sections were obtained including a section of the healthy uninvolved testicular parenchyma, fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Tumor diameter was defined as the largest dimension measured by the pathologist before specimen fixation. Multifocality was defined as a distinct tumor focus with a diameter $\geq$ 1 mm, separable from the main tumor mass. The following pathological features were analyzed for each orchiectomy specimen: tumor size of the main tumor mass, multifocality of the lesions, testicular vascular invasion (tumor cells within lymphatic or venous vessels at the testicular level), rete testis invasion (tumor cells in contact with or between the ducts of the rete testis), and TIN (microinvasive tumor characterized by a single or small groups of malignant germ cells scattered within the normal intratubular testicular parenchyma but not forming a macroscopically overt lesion).

Statistical Analysis

All statistical analyses were completed using SPSS version 19 software (SPSS Inc, IBM Corp, Somers, NY). The qualitative data were tested using the χ^2 test or Fisher exact test as appropriate, and the continuous variables, presented as median, were tested using a Mann-Whitney U test.

Univariate logistic regression was performed to identify the association between pathological variables and multifocality. Multivariate logistic regression analyses were carried out to identify variables for predicting clinical stage II to III and pathological stage $\geq$ pT2.

For all statistical comparisons, significance was considered as $P < .05$.

Results

The baseline characteristics of all subjects are listed in Table 1. Of the 126 patients, 103 (82.0%) had clinical stage I cancer at presentation and 23 (18.0%) had clinical stage II to III cancer. The median patient age was 33 (interquartile range [IQR], 22-68). The median tumor diameter was 3.5 (IQR, 1.2-10.8). Among all, lymphovascular invasion was present in 48 (38%), and rete testis invasion in 34 (27%) cases, and there were 17 orchiectomy specimens (13.5%) containing more than 50% embryonal carcinoma. Among patients with multifocality, 25 (75.7%) had only

Table 1 Baseline Characteristics of Subjects

Characteristic	Value
Patients, n	126
Median Age (IQR), Years	33 (22-68)
Median Tumor Size (IQR), cm	3.5 (1.2-10.8)
Histology, n (%)	
Seminomatous	76 (60.3)
Nonseminomatous	50 (39.7)
Clinical Stage, n (%)	
I	103 (82.0)
II and III	23 (18.0)
Serum Markers	
LDH, n (%)	
<1.5, IU/L	103 (82.0)
1.5-10, IU/L	23 (18.0)
>10, IU/L	–
Median B-HCG (IQR), mIU/L	0.7 (2.0-198000)
Median AFP (IQR), ng/mL	0.65 (0.2-166750)
Tumor Size, cm	
0-1	6 (4.76)
1.1-2	22 (17.46)
2.1-3	26 (20.63)
3.1-4	21 (16.67)
>4	50 (39.68)
Pathological T Stage, n (%)	
pT1	79 (63.0)
pT2	42 (34.0)
pT3	5 (3.0)
Multifocality, n (%)	33 (26.19)
TIN, n (%)	46 (36.50)

Abbreviations: AFP = alpha fetoprotein; B-HCG = Beta Human Chorionic Gonadotropin; IQR = interquartile range; LDH = lactate dehydrogenase; TIN = testicular intraepithelial neoplasia.

1 additional distinct tumor focus with a median diameter of 1.94 cm (range, 0.6-5) and 8 (24.2%) had 2 additional distinct tumor foci with a median diameter of 1.7 cm (range, 0.6-4).

Subjects with multifocality had larger primary tumor lesions. The median size of the primary tumor mass was 3.7 cm (range, 0.5-12) in multifocality and 3.0 cm (range, 0.6-8.0) in monofocality respectively ($P < .05$).

According to tumor size of the main tumor mass with a diameter $\leq$ 1 cm, 1.1 to 2 cm, 2.1 to 3 cm, 3.1 to 4 cm, and $>$ 4 cm, multifocality was present in 2 (1.6%), 9 (7.1%), 9 (7.1%), 4 (3.1%), and 9 (7.1%) cases, respectively and TIN in 0 (0%), 10 (7.9%), 6 (4.7%), 9 (7.1%), and 20 (16.6%) cases, respectively. The prevalence of multifocality and TIN was decreased in the presence of a smaller main tumor mass ($\leq$ 1 cm) compared with tumors between 1.1 and 2.0 cm ($P < .05$) and increased when the index mass tumor diameter was $\geq$ 2 cm (P trend $< .05$; Figure 1).

No association was found between tumor histology and multifocality ($P = .95$) or TIN ($P = .54$) in χ^2 testing. In univariate logistic regression analysis, multifocality was not significantly associated with tumor size $\geq$ 4 cm and Rete Testis Invasion and neither with lymphovascular invasion and percentage of embryonal carcinoma $>$ 50% in seminoma and nonseminoma tumors, respectively (Table 2). The presence of necrosis was not associated with the presence of multifocal tumors, either for seminoma or for nonseminoma tumors. In multivariate logistic regression analysis, multifocality was not demonstrated to be an adverse pathological feature of clinical stage II to III ($P = .23$; c-index = 0.60) or pathological stage $\geq$ pT2 ($P = .30$; c-index = 0.88) when included in a model with tumor size $\geq$ 4 cm and Rete Testis Invasion in seminoma tumors and neither of clinical stage II to III ($P = .36$; c-index = 0.67) or pathological stage $\geq$ pT2 ($P = .20$; c-index = 0.72) when included in a model with lymphovascular invasion, and percentage of embryonal cancer $\geq$ 50% in nonseminoma tumors (Table 3).

Discussion

Standard therapy for testicular cancer is radical orchiectomy. However, in men with bilateral tumors, removing the 2 gonads invariably results in infertility and testosterone deficiency. To avoid these consequences, several groups have offered organ-sparing surgery, consisting of tumor enucleation with preservation of the normal-appearing parenchyma. In synchronous bilateral testicular tumors, metachronous contralateral tumors or in a tumor in a solitary testis with normal preoperative testosterone levels, organ-preserving surgery can be performed when the tumor volume is $<$ 30% of the testicular volume and surgical rules are respected. In those cases, the rate of associated TIN is high (at least up to 82%), and all patients must be treated with adjuvant radiotherapy (16-20 Gy) at some point.[7] The entity of intratubular germ cell neoplasia (IGCNU), first described by Skakkebaek[11] as carcinoma in situ of the testis (TIN), has been incorporated into the spectrum of germ cell tumors. TIN is a preinvasive stage of TGCT characterized by replacement of the normal-appearing germ cells in the seminiferous tubules by neoplastic cells. TIN is frequently observed in the testicular parenchyma adjacent to invasive cancer, and a prospective study of patients with IGCNU show that 50% of patients developed invasive germ cell tumors within 5 years of diagnosis.[9,12] Furthermore, multifocality could be a finding in TGCT cases and in those with seminomatous histology, as reported in a recent study.[9] Recent data suggest that additional invasive tumors outside the index mass might be present in up to 63% of men considered to be potential candidates for organ-sparing surgery.[9] To determine the prevalence of TIN and multifocality in TGCT cases, we analyzed orchiectomy specimens from 126 consecutive patients treated for TGCT in our institution. Compared with a recent work of Ehrlich et al[9] in which the multifocality was defined as 1 of 4 distinct pathological entities, including distinct tumor focus conspicuously separable from the main tumor mass, microinvasive tumor characterized by a single or small groups of malignant germ cells scattered within the normal interstitial parenchyma, extra tumor vascular invasion, and rete testis invasion by pagetoid tumor spread, we have preferred to consider multifocality as a distinct tumor focus separable from the main tumor mass with a diameter $\geq$ 1 mm and TIN as a microinvasive tumor characterized by a single or small groups of malignant germ cells scattered within the normal intratubular testicular parenchyma but not forming a macroscopically overt lesion. In fact, vascular lymphatic invasion and rete testis involvement are not traditionally considered criteria for multifocality but together with percentage of embryonal carcinoma

Table 2 Univariate Logistic Regression Between Pathological Variables and Multifocality in Seminoma and Nonseminoma Testis Cancer

Variable	OR (95% CI)	*P*
Seminoma		
Tumor size, $\geq$4 cm versus $<$4 cm	1.13 (0.57-2.25)	.72
Rete testis invasion, yes versus no	1.43 (0.78-2.61)	.25
Nonseminoma		
Lymphovascular invasion, yes versus no	0.46 (0.18-1.15)	.09
Percentage of embrional carcinoma $>$50%, yes versus no	0.1 (0.0-0.2)	.99

Figure 1 Prevalence (%) of Multifocality and TIN According to Diameter of the Main Tumor Mass

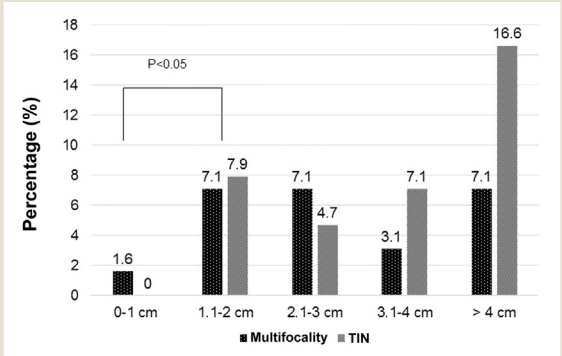

Table 3 Multivariate Logistic Regression Analysis Between Pathological Risk Factors of Advanced Clinical Stage and Pathological Stage ≥ pT2

Variable	Clinical Stage II to III		Pathological Stage ≥pT2	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Nonseminoma				
Multifocality, yes versus no	0.49 (0.10-2.28)	.36	3.17 (0.53-18.73)	.20
Lymphovascular invasion, yes versus no	1.83 (0.43-7.72)	.04	4.28 (1.05-17.44)	.04
Percentage of embrional carcinoma > 50%, yes versus no	5.38 (0.93-31.13)	.04	7.18 (1.31-39.36)	.02
c-Index of overall model	0.60		0.88	
Seminoma				
Multifocality, yes versus no	0.40 (0.07-2.24)	.23	0.62 (0.48-1.55)	.30
Rete testis invasion, yes versus no	7.64 (1.12-10.91)	.02	2.23 (1.06-4.68)	.03
Tumor size, ≥4 cm versus <4 cm	3.11 (0.81-11.92)	.03	2.60 (1.25-5.40)	.01
c-Index of overall model	0.67		0.72	

> 50% and tumor size (> 4 cm) are established risk factors associated with the risk of systemic relapse.[13,14] According to our definition, multifocality was identified in 33 (26.1%) and TIN in 45 (35.7%) of 126 orchiectomy specimens.

According to the findings of our study, multifocality did not correlate with the histological subtype, in particular, seminomatous histology as previously described[9] or with other adverse clinical and pathological variables. Moreover, if a new pathological feature can be considered potentially efficient, it should demonstrate similar results of pre-existing models and it should be compared with them.[15] In fact, multifocality was not demonstrated to be an adverse pathological factor associated with clinical stage II to III and pathological stage ≥ pT2.

Despite these results, multifocality always has been considered an adverse pathological feature in several urological tumors, like bladder and kidney cancer, but its clinical effect in TGCT in a long-term follow-up setting is not well understood.

The prevalence of multifocality and TIN was decreased in the presence of a smaller main tumor lesion (≤ 1 cm) and increased when the index mass tumor diameter was between 1.1 and 2 cm or more. The current guidelines of the European Urological Association and the European Germ Cell Cancer Consensus Group recommend organ-sparing surgery, in selected nonmetastatic patients, with tumors ≤ 2 cm in diameter.[7,13,16] In our analysis, 33 patients (26.19%) when the sample size is 126 patients demonstrated multifocal cancer and would not be candidates for an organ-preserving approach. It should be taken into account that the oncological efficacy of organ-sparing surgery for testicular cancer depends entirely on the ability of the surgeon to eradicate the invasive component of the disease. Based on our results, we suggest to perform a testis-sparing surgery in patients with tumor diameter ≤ 1 cm. This approach could respect the long-term oncological safety of testicular cancer and reduce the need of irradiation to the retained testicular parenchyma. Several limitations should be considered when interpreting the results of our study, which are related mainly to its retrospective design and to the lack of available follow-up data. In fact, the small sample size and the absence of follow-up data might have limited the interpretation of our results about the association between multifocality and risk of relapse.

Second, the prevalence of TIN might have been underestimated because of the lack of octamer-binding transcription factor 3/4 immunochemistry analysis or the possibility that TIN might have been enveloped by the main tumor mass, because we also considered diameter of 3.5 cm (range, 1.2-10.8 cm).

Finally, our cohort consisted of unselected consecutive patients who had undergone radical orchiectomy for TGCT, and testis-sparing surgery can be performed in carefully selected patients with solitary tumor foci with a decreased likelihood of concomitant tumor lesion. However, even with these limitations, our study findings might be considered in the choice of treatment in patients who are candidates for testis-sparing surgery to obtain better oncological control of the disease.

Conclusions

Our study showed that the prevalence of multifocality testicular cancer and TIN were decreased in the presence of a smaller main tumor mass (≤ 1 cm) and increased when the index mass tumor diameter was ≥ 1.1 cm. Furthermore, these findings should be considered even in subjects potentially eligible for testis-sparing surgery.

Clinical Practice Points

- Based on the findings of the current study, we suggest particular attention, even for tumor masses < 2 cm, during testis-sparing surgery.
- Although the pathological behavior of multifocality lesions is not well understood, its prevalence is considerable even in lesions between 1.1 and 2 cm, and in TIN.
- Patients potentially eligible for testis-sparing surgery should be advised of these events.

Disclosure

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

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