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ORIGINAL ARTICLE

Testicular ultrasonographic features predict future risk for bilateral testicular germ cell tumour: A long-term single centre follow-up study

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Abstract

Background: Bilateral testicular germ cell tumours (B-GCT) are rare, with an incidence of 2–5%, and can be classified as synchronous (sB-GCT) or metachronous (mB-GCT). Our study aimed to identify clinical, biochemical, and radiological risk factors for mB-GCT in a cohort of patients with GCT at a single tertiary referral centre.

Methods: This retrospective case-control study included patients with GCT referred to Policlinico Umberto I—Sapienza University of Rome, from 2005 to 2023. We evaluated clinical history, testicular ultrasound features, hormone levels, semen analysis, histological characteristics, staging, and treatments. mB-GCTs were compared with unilateral GCT patients with a follow-up longer than the median time-to-onset of the second tumour.

Results: Of 319 patients, 52 experienced B-GCT, with a median time-to-onset of the second tumour of 62 months (range: 8–229). The mB-GCT group showed higher gonadotropin levels (FSH 13.6mUI/mL vs. 7.4mUI/mL, $p < 0.001$; LH 6.6mUI/mL vs. 3.9mUI/mL, $p = 0.004$), lower sperm concentration (27×10^6/ejaculate vs. 78×10^6/ejaculate, $p = 0.009$), smaller residual testis volume (10.4 mL vs. 16.3 mL, $p < 0.001$), more inhomogeneous echotexture [57.5% vs. 14%, $p < 0.001$], and presence of microlithiasis (75% vs. 19.5%, $p < 0.001$). Kaplan–Meier curves confirmed that ultrasound features of the residual testis increased the cumulative risk of developing a second tumour. Microlithiasis was a strong independent predictor (OR 30.712, 95% CI 3.357–280.942, $p = 0.002$).

Conclusions: Histological features of the first tumour or its treatment do not influence the onset of a second tumour. However, low residual testis volume,

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inhomogeneous echotexture, and microlithiasis significantly increase this risk. A comprehensive evaluation of the residual testis at baseline is essential for developing a personalised surveillance programme in GCT survivors, with regular ultrasound follow-up recommended beyond the conventional 5-year limit.

KEYWORDS
bilateral testicular tumours, microlithiasis, mixed germ cell tumours, seminoma, testicular ultrasound

1 | INTRODUCTION

Testicular cancer is the most common cancer among young males aged 15–40, with incidence rates steadily increasing over the past two decades.[1,2] Testicular germ cell tumours (GCTs) represent over 98% of all testicular cancers.[3,4] Bilateral GCTs (B-GCT) are rare, with an incidence of 2–5%.[5,6] B-GCTs can manifest as synchronous (sB-GCT) or metachronous (mB-GCT) when the second tumour develops after 3 months from the first diagnosis,[5] with mB-GCT being more prevalent than sB-GCT.[7] The interval between tumours can range from months to several years, sometimes exceeding 20 years.[8,9]

Testicular ultrasound plays a crucial role in the early diagnosis of GCT[10,11] and should be included in follow-up protocols, as stated in some European National Guidelines.[12–14] However, current International Guidelines do not uniformly advocate for ultrasound surveillance due to the limited evidence on this topic.[15,16] The risk factors associated with developing a second tumour and the optimal strategy and duration for US surveillance remain unclear. This study aims to compare mB-GCT patients with a cohort of long-term surveillance unilateral-GCT patients to identify clinical, biochemical, and ultrasound risk factors associated with the occurrence of a second malignant tumour.

2 | MATERIALS AND METHODS

2.1 | Population and study design

This retrospective single-centre case-control study involved patients diagnosed with GCT and referred to the *Testis Unit* of Policlinico Umberto I, Rome, between January 2005 and June 2023. Exclusion criteria included monorchid patients for non-neoplastic reasons, burned-out tumours, patients lost to follow-up, and incomplete records.

Medical records were reviewed, and data on demographics, laboratory parameters, radiological findings, histological characteristics, staging, and treatment were collected. Contralateral testis biopsy was not routinely performed.[15] The follow-up duration (from orchiectomy to the last ultrasound) was calculated. Missing data were supplemented through telephone interviews.

B-GCTs were classified as sB-GCT or mB-GCT. Regarding sB-CGT, the larger tumour was considered to be the one that appeared first. The median time-to-onset of mB-GCT was calculated. Patients with mB-GCT were compared with unilateral GCT patients with follow-up beyond the median time-to-onset of the second tumour. All patients provided written informed consent. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Policlinico Umberto I (Rif. CE 6478 Prot. 1038).

2.2 | Procedures

2.2.1 | Hormonal and semen analysis

Hormonal and semen analyses were all conducted in the Laboratory of our Department and obtained after orchiectomy and before any oncological treatment. Serum levels of FSH, LH, 17β-estradiol, total testosterone, prolactin, and inhibin B were measured using standard laboratory techniques as previously reported.[17,18] Semen samples were analysed according to WHO criteria[19] and, for previous samples, the 1999 WHO criteria.[20] Evaluated variables included semen volume, concentration ($\times 10^6$/mL), total sperm count ($\times 10^6$/ejaculate), total motility (%), and morphology (% abnormal forms). Total motility was selected over progressive motility to mitigate differences in motility assessment.[21]

2.2.2 | Ultrasound examinations

Ultrasound examinations were performed using a Philips IU22 unit by two operators with expertise in scrotal sonography (A.M.I., C.P.) and reviewed by a third operator (M.T.). Patients underwent at least two ultrasounds yearly for the first 5 years of follow-up and one ultrasound per year thereafter, as per *Testis Unit* clinical practice. Parameters assessed included testicular volume, echotexture, echogenicity, and the presence of testicular microlithiasis[11,22] (Figure 1). Testicular volume was considered reduced if <12 mL.[22]

2.2.3 | Histological features

Histological reports provided tumour dimension, testicular volume, presence of GCNIS, infiltration details, stage, and metastases (AJCC criteria).[23]

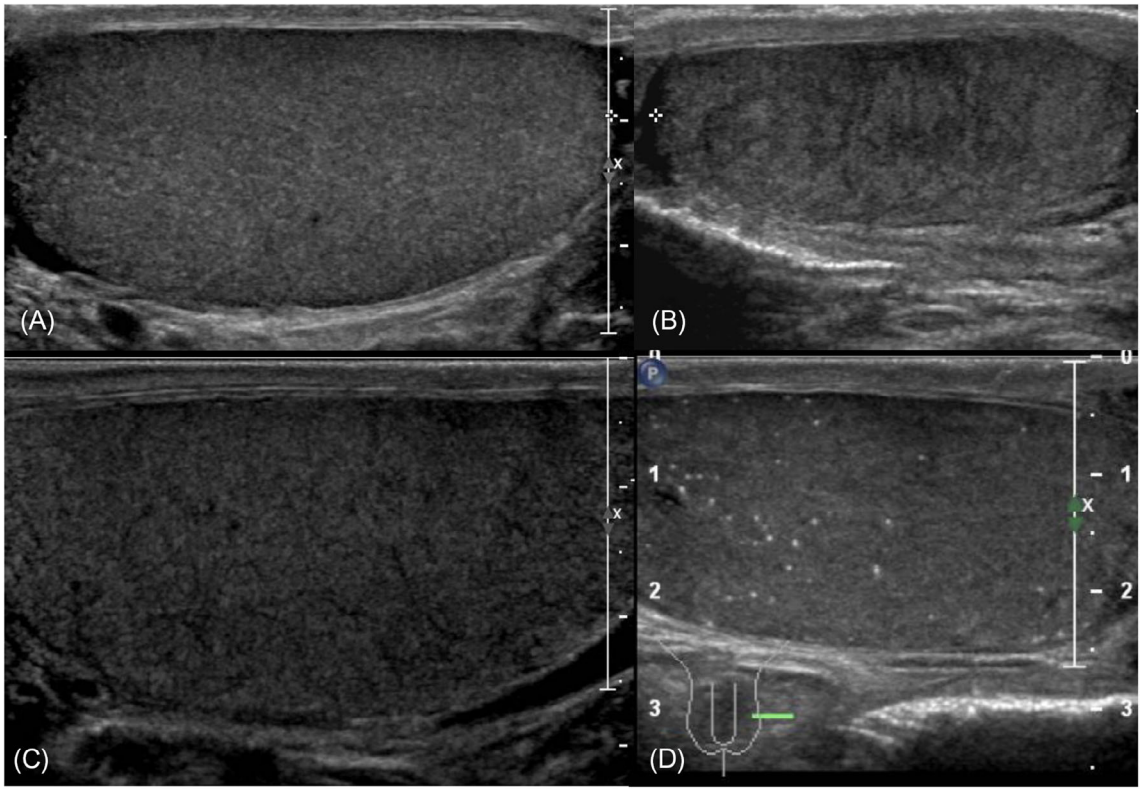

FIGURE 1 Ultrasound features evaluated. (A) Homogeneous echotexture; (B) inhomogeneous echotexture; (C) reduced echogenicity; (D) presence of testicular microlithiasis. Testicular microlithiasis was initially graded as absent or isolated, mild (at least five microcalcifications per ultrasound scan), moderate (more than 10 per ultrasound scan), or starry sky (homogenous presence of high-density microcalcifications in the testis). However, exploratory data analysis revealed no significant differences between these groups in relation to the prediction of seminal, hormonal parameters, or the risk of developing a mB-TGCT, therefore it was dichotomized into two groups: absence or presence of testicular microlithiasis.

2.2.4 | Treatment

Postorchiectomy therapeutic procedures were recorded, including active surveillance, chemotherapy (carboplatin or bleomycin, etoposide, and platinum [BEP]), prophylactic radiation therapy, and retroperitoneal lymph node dissection (RPLND).

2.3 | Statistical analysis

Outcome measurements were assessed for normality using the Shapiro–Wilk test. Nonparametric tests were employed when parametric test assumptions were violated. Values were expressed as median and interquartile range (IQR). Group comparisons utilized the Mann–Whitney test for continuous variables or odds ratios (OR) with 95% confidence intervals (CIs) for categorical variables. As appropriate, the chi-square or Fisher's exact tests were applied for categorical variables. The Wilcoxon signed-rank test was used to compare paired samples or repeated measures when the data were not normally distributed. Kaplan–Meier survival analysis evaluated the cumulative risk of second tumour occurrence, with pairwise log-rank comparisons to identify risk-enhancing features. Logistic regression analysis assessed the association between independent variables and the second tumour, with bootstrap resampling ($N = [2000]$) to obtain robust estimates. Statistical significance was set at $p < 0.05$. Analyses were performed using SPSS Statistics version 27.0 (IBM SPSS Statistics Inc.).

3 | RESULTS

3.1 | Overall population

Between January 2005 and June 2023, 403 patients with GCT were referred to the *Testis Unit*. According to enrolment criteria, 84 patients were excluded (10 burned-out tumours, 1 monorchid, 38 incomplete data, and 35 lost at follow-up, Figure 2), and 319 remained. Among them, 174 patients were diagnosed during an ultrasound at our facility, while 145 patients were acquired during follow-up. The median age at diagnosis was 32 years (range 16–60), with a median follow-up time of 53 months (range 6–314). All the patients survived except for one with a poor prognosis due to visceral metastasis. Seminoma was the predominant histological type (61.8%; Figure S1). Characteristics of the overall population are detailed in Table S1.

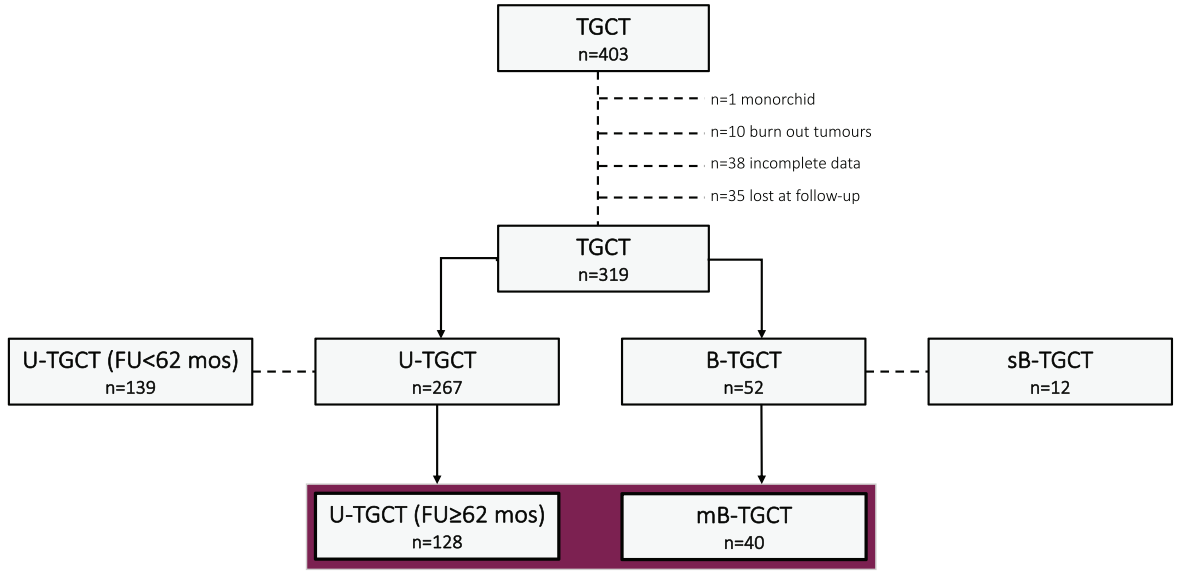

FIGURE 2 Flow chart of the study. B-TGCT, bilateral testicular germ cell tumour; FU, follow-up; mB-TGCT, metachronous bilateral testicular germ cell tumour; mos, months; sB-TGCT, synchronous bilateral testicular germ cell tumour; TGCT, testicular germ cell tumour; U-TGCT, unilateral testicular germ cell tumour.

3.2 | Bilateral tumours

B-GCTs were identified in 52 patients, 12 sB-GCT and 40 mB-GCT (Table 1). Seminoma was the predominant histology for both groups, with 32.7% of cases exhibiting discordant histology between the first and second tumours (Figure 3).

The median time-to-onset of the second tumour for mB-GCT was 62 months (range: 8–229; Table 2). In the group of mB-GCT, the size of the second tumour was significantly smaller compared with the first tumour: 1.0 (0.9;2.6) vs. 3.2 (1.9;4.6) cm, $p < 0.001$. Only 8 tumours (20%) were palpable. No substantial ultrasound changes (in volume and structural characteristics of the surviving testicle) were observed during the follow-up (data not shown).

The distribution of second tumour occurrence time was as follows: ≤2 years, 7 patients (17.5%); 2–5 years, 13 patients (32.5%); 5–10 years, 8 patients (20%); ≥10 years, 12 patients (30%).

3.3 | mB-GCT vs. U-GCT

Patients with follow-up ≥62 months served as the control group (U-GCT, $n = 128$). No significant differences were observed in age, BMI, medical history, histological features, stage, or basal serum tumour markers between mB-GCT and U-GCT (Table 2). No difference was observed between the two groups based on the treatment adopted. Specifically, a Cox regression showed no difference between the two groups, even based on the number of therapy cycles (data not shown). However, patients with mB-GCT exhibited higher FSH (13.6 mUI/mL vs. 7.4 mUI/mL, $p < 0.001$) and LH (6.6 mUI/mL vs. 3.9 mUI/mL, $p = 0.004$) levels, lower INHB levels (58.1 pg/mL vs. 78.5 pg/mL, $p = 0.035$) and poorer semen concentration (5.5×10^6/mL vs. 22.0×10^6/mL, $p = 0.012$ and 27×10^6/ejaculate vs. 78.0×10^6/ejaculate, $p = 0.009$). In the mB-GCT group, a higher proportion of azoospermic patients was also reported ($p = 0.004$; Table 2).

Basal ultrasound findings did not differ, but significant differences were observed in residual testicle ultrasound evaluation: mB-GCT patients had lower residual testicular volume ($p < 0.001$), more inhomogeneous echotexture ($p < 0.001$), and higher prevalence of testicular microlithiasis ($p < 0.001$) compared with U-GCT (Table 2).

Kaplan–Meier analysis demonstrated a higher cumulative risk of second tumour development in patients with reduced testicular volume (χ^2 (1) 14.379, $p < 0.001$; Figure 4A), inhomogeneous echotexture (χ^2 (1) 20.698, $p < 0.001$; Figure 4B), and testicular microlithiasis (χ^2 (1) 33.334, $p < 0.001$; Figure 4C).

In the logistic regression model incorporating these parameters along with testicular function markers, only testicular microlithiasis remained significant ($p = 0.002$). For a patient with a previous testicular tumour, the presence of testicular microlithiasis in the residual testicle increases the risk (OR) of developing a second tumour by 30.712 times, 95% CI (3.357–280.942; Table 3).

4 | DISCUSSION

In a large retrospective from a tertiary referral centre study, we revealed that the development of metachronous testicular tumours can extend over many years, highlighting the necessity of continued surveillance. Patients with mB-GCT exhibit elevated gonadotropin levels and poorer seminal characteristics compared with those with unilateral GCT. Primary risk factors for second tumour development are associated with ultrasound characteristics of the residual testis,

TABLE 1 Characteristic of overall TGCT population and divided into mB-TGCT and sB-TGCT subgroups.

	B-TGCT($n = 52$)	mB-TGCT($n = 40$)	sB-TGCT($n = 12$)	p-value (mB vs. sB)
Age at diagnosis (years)	31 (17–42)	29 (17–41)	35 (23–42)	0.018
BMI (kg/m^2)	24.5 (23.7;27.8)	24.5 (23.7;26.2)	24.4 (21.8;28.6)	0.656
FU time (months)	62 (8–229)	62 (8–229)	–	–
Risk factors				
Cryptorchidism in the first affected testis	9 (17.3)	8 (20)	1 (8.3)	0.360
Cryptorchidism in the second affected testis	7 (13.5)	4 (10)	3 (25)	0.220
Bilateral cryptorchidism	3 (5.8)	2 (5)	1 (8.3)	0.587
Smoke	19 (36.6)	10 (25)	9 (75)	0.003
Infertility	5 (9.6)	5 (12.5)	–	0.355
First tumor features				
Seminoma	37 (71.2)	28 (70)	9 (75)	0.523
Nonseminoma	15 (28.8)	12 (30)	3 (25)	0.523
Diameter (cm)	2.5 (1.9;4.6)	2.5 (1.9;4.6)	3.0 (2;4.1)	0.610
Multifocality	18 (34.6)	12 (30)	6 (50)	0.406
pT				
pT1	33 (63.5)	26 (65)	7 (58.3)	0.462
pT2	18 (34.6)	13 (32.5)	5 (41.7)	0.399
pT3	1 (1.9)	1 (2.5)	–	0.769
Metastases				
Diagnosis	4 (7.7)	2 (5)	2 (16.7)	0.224
Follow-up	4 (7.7)	4 (10)	–	0.338
Clinical Stage				
Stage I	48 (92.3)	38 (95)	10 (83.3)	0.183
Stage II	3 (5.8)	1 (2.5)	2 (16.7)	0.065
Stage III	1 (1.9)	1 (2.5)	–	0.769
Treatment				
Active surveillance	16 (30.8)	11 (27.5)	5 (41.7)	0.277
Chemotherapy - 1°line	23 (44.2)	17 (42.5)	6 (50)	0.646
Carboplatin	10 (19.2)	8 (20)	2 (16.7)	0.367
BEP	13 (25)	9 (22.5)	4 (33.3)	0.344
Chemotherapy - 2° line	2 (3.8)	2 (5)	–	0.588
Radiotherapy	13 (25)	12 (30)	1 (8.3)	0.125
RPLND	3 (5.8)	2 (5)	1 (8.3)	0.551
Semen analysis	**($n = 36$)**	**($n = 27$)**	**($n = 9$)**	
Normozoospermia	12 (33.3)	11 (27.5)	1 (11.1)	0.108
Oligozoospermia	15 (41.7)	9 (22.5)	6 (66.7)	0.079
Azoospermia	9 (25)	7 (17.5)	2 (22.2)	0.602
Hormones	**($n = 27$)**	**($n = 17$)**	**($n = 10$)**	
FSH (mIU/mL)	19.2 (8.2;31)	13.6 (8.2;35)	21.9 (7.9;29.7)	0.941
LH (mIU/mL)	6.7 (4.1;13.4)	6.6 (3.7;14)	6.8 (4.3;12.2)	0.853
Testosterone (nmol/L)	15.8 (11.9;21.5)	16.5 (12.3;24.9)	14.6 (3.3;20.2)	0.180
Serum tumour markers				
α-FP	7 (13.5)	5 (12.5)	2 (16.7)	0.571
β-hCG	8 (15.4)	6 (15)	2 (16.7)	0.657

Note: Regarding sB-CGT, the larger tumour was considered to be the one that appeared first. Values are expressed as median and interquartile ranges and in number and percentage. Comparisons between two groups were performed at each time point using the Mann–Whitney *U* test, Chi-square test, or Fisher's exact test, as appropriate.

Abbreviations: BMI, body mass index; BEP, bleomycin, etoposide, and platinum; RPLND, retroperitoneal lymph node dissection.

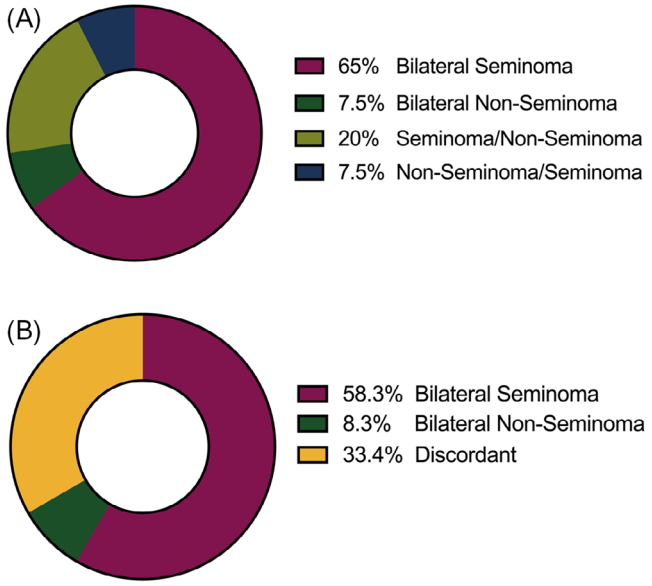

FIGURE 3 Pie chart of histology concordance in bilateral tumours. (A) Metachronous tumours (mB-GCT). (B) Synchronous tumours (sB-GCT).

including reduced testicular volume, inhomogeneous echotexture, and testicular microlithiasis, but not with the first histotype, stage or treatment. The prompt recognition of these ultrasound features should help identify those high-risk patients who need stricter and longer surveillance strategies.

The observed incidence of B-GCT in our cohort (16.3%) is notably higher than rates reported in the literature (2–5%).[5] However, caution must be exercised in interpreting this number, as our population consists only of individuals undergoing regular testicular ultrasounds within our institution. Consequently, the observed incidence may not be directly comparable to that reported in larger cohorts and registry studies. Nonetheless, the overall incidence of GCTs, including bilateral cases,[24] has been increasing in recent years,[2] highlighting the need for further investigation into this phenomenon.

Consistent with previous studies, metachronous tumours were more prevalent than synchronous ones, and mB-GCT patients tended to be younger than sB-GCT.[5] According to the literature, histological disagreement between the first and second tumours was observed in about one-third of cases, irrespective of tumour synchronicity.[5,25,26]

The median time-to-onset of the second GCT in our cohort of mB-GCT population (62 months) aligns with existing literature,[5,24,27] emphasising the importance of long-term surveillance. Only 50% of our patients developed a second tumour within a 5-year follow-up, while a full 30% did so after 10 years.[28] Moreover, the second tumours were significantly smaller than the first ones, with only a small percentage being palpable. This finding underscores the critical importance of regular ultrasound monitoring to identify lesions at an early stage, which may not yet be palpable, thereby facilitating earlier diagnosis, timely intervention, and a more conservative surgical approach.[10,29,30] Unfortunately, current international guidelines still do not include testicular ultrasound in GCT follow-up programs.[15,16] We believe ultrasound surveillance should be emphasised in clinical practice guidelines, as already done by some European National Guidelines.[12–14] Moreover, considering the young age of affected subjects and that the median time-to-onset of the second tumour exceeds 5 years, ultrasound surveillance should be extended beyond the standard oncological follow-up.

Interesting data emerged when comparing the mB-GCT and U-GCT groups. At univariate analysis, no significant difference resulted between the anamnestic risk factors explored, aside from the fact that patients with mB-GCT were younger than U-GCT.[28,31–33]

The existing literature on histological types related to the risk of developing a metachronous tumour is inconsistent. Research conducted before the widespread use of cisplatin-based chemotherapy showed a higher risk in nonseminoma,[34,35] while in the cisplatin era, some studies have suggested the opposite trend.[27,36–39] Our study did not find any difference in histological types among mB-CGT and U-GCT, which is consistent with other reports.[40,41] Seminomas were the most frequent histotype in both groups, as previously reported.[5]

No group differences were found in distinct histological features or the presence of distant metastases at diagnosis or follow-up. Instead, we observed a higher frequency of albuginea infiltration in U-GCT patients, which has been identified as a marker of distant metastasis of testicular cancer.[42] This data reinforces the de novo origin of the second tumour. To the best of our knowledge, this is the first study that considers all histological features described in a pathological report, enabling a more precise determination of whether the characteristics of the initial tumour can influence the onset of the second. Based on our results, the histology of the primary tumour does not appear to affect the onset of an mB-GCT,[6,34] contrary to data reported for recurrences of metastatic disease.[43,44]

No difference has been found considering adjuvant treatments adopted, as already described.[24,28,45–47] However, several reports have suggested a decreased risk of contralateral disease in patients treated with platinum-based chemotherapy,[26,27,39,41,48–54] with some also observing a dose-dependent effect.[36,40,55] This effect is attributed to the ability of platinum-based chemotherapy to penetrate the blood–testis barrier and eliminate potential GCNIS. However, evidence seems to suggest that such a protective effect may not be consistent in stage I tumours,[45] likely due to a lower efficacy of carboplatin compared with cisplatin on GCNIS.[56] The predominance of stage I tumours in our population, could explain the findings and also the reasons for a higher number of bilateral cases compared with previous reports.[5,6]

The most intriguing findings stem from the function and ultrasound characteristics of the residual testicle. Impaired testicular function is commonly considered a risk factor for GCTs for the link between gonadal dysgenesis and testicular tumourigenesis.[57,58] However, relying solely on "infertility" as anamnestic data fails to identify the "at-risk" population. In our cohort, the history of infertility was comparable between the two groups, likely influenced by the younger age of mB-TGCT patients, who have undergone fewer attempts to conceive. Conversely, lower spermatozoa concentration and higher gonadotropin levels were observed in the mB-GCT group. Several

TABLE 2 Comparison between mB-GCT and U-GCT: Risk factors, tumour features, stage, serum tumour markers, treatment, hormones, semen analysis, and ultrasound features.

	mB-TGCT($n = 40$)	u-TGCT($n = 128$)	*p*-value
Age at diagnosis (years)	29 (17-41)	31 (16-60)	0.031
BMI (kg/m^2)	24.5 (23.7;26.2)	24.7 (22.8;26.9)	0.455
FU time (months)	62 (8-229)	84 (62.2;110.5)	0.035
Risk factors			
Cryptorchidism in the first affected testis	8 (20)	19 (14.8)	0.583
Cryptorchidism in the second affected testis	4 (10)	9 (7)	0.423
Bilateral cryptorchidism	2 (5)	4 (3.1)	0.550
Smoke	10 (25)	53 (41.4)	0.188
Infertility	5 (12.5)	28 (21.8)	0.251
Tumour features			
Seminoma	28 (70)	77 (60.2)	0.262
Nonseminoma	12 (30)	51 (39.8)	0.262
Diameter	2.5 (1.9;4.6)	1.7 (1.2;2.9)	0.599
Multifocality	12 (30)	31 (24.2)	0.468
GCNIS	25 (62.5)	74 (58)	0.329
pT			
pT1	26 (65)	82 (64)	0.914
pT2	13 (32.5)	40 (31.2)	0.882
pT3	1 (2.5)	6 (4.7)	0.472
Tumour infiltration (AJCC)			
Rete testis	16 (40)	41 (32)	0.281
Tunica albuginea	10 (25)	60 (46.9)	0.033
Tunica vaginalis	1 (2.5)	6 (4.7)	0.490
Vessels	10 (25)	34 (26.5)	0.901
Lymphatic vessels	7 (17.5)	23 (18)	0.974
Epididymis	2 (5)	6 (4.7)	0.594
Funiculus	1 (2.5)	6 (4.7)	0.498
Metastasis			
Diagnosis	2 (5)	13 (10.1)	0.257
Follow-up	4 (10)	17 (13.2)	0.406
Clinical stage			
Stage I	38 (95)	115 (89.8)	0.526
Stage II	1 (2.5)	12 (9.4)	0.306
Stage III	1 (2.5)	1 (0.8)	0.421
Positive serum tumour markers			
α-FP	5 (12.5)	16 (12.5)	0.454
β-hCG	6 (15)	25 (19.5)	0.822
Treatment			
Active surveillance	11 (27.5)	37 (28.9)	0.864
Chemotherapy - 1°line	17 (42.5)	71 (55.5)	0.152
Carboplatin	8 (20)	27 (21.1)	0.312
BEP	9 (22.5)	45 (35.1)	0.320
Chemotherapy - 2°line	2 (5)	10 (7.8)	0.422
Radiotherapy	12 (30)	23 (17.9)	0.102
RPLND	2 (5)	10 (7.8)	0.366

(Continues)

TABLE 2 (Continued)

	mB-TGCT($n = 40$)	u-TGCT($n = 128$)	*p*-value
Semen analysis	**($n = 27$)**	**($n = 99$)**	
Volume (mL)	3.5 (2.2;4.1)	3.2 (2.6;5)	0.182
pH	7.5 (7.4;7.5)	7.5 (7.4;7.6)	0.589
Sperm concentration (10^6/mL)	5.5 (0.1;32.7)	22 (7;48)	0.012
Total sperm number (10^6/ml)	27 (0.3;118)	78 (24.3;173)	0.009
Total Motility (%)	30 (0;50)	40 (22.5;50)	0.220
Atypical morphology (%)	83 (71;92)	88 (78;94.7)	0.105
Normozoospermia	11 (27.5)	59 (59.6)	0.081
Oligozoospermia	9 (22.5)	35 (35.3)	0.845
Azoospermia	7 (17.5)	5 (5)	0.004
Hormones	**($n = 17$)**	**($n = 83$)**	
FSH (mIU/mL)	13.6 (8.2;35)	7.4 (3.5;10.6)	<0.001
LH (mIU/mL)	6.6 (3.7;14)	3.9 (2.7;5.5)	0.004
Testosterone (nmol/L)	16.5 (12.3;24.9)	18.3 (13.3;22.6)	0.579
Estradiol (pg/mL)	28.5 (21.5;34)	28 (21;44)	0.702
Inhibin B (pg/mL)	58.1 (4.5;76.8)	78.5 (44.7;102.2)	0.032
PRL (ng/mL)	10.8 (7.7;16.2)	8.9 (6.5;11.9)	0.299
Ultrasound features			
First affected testis	**($n = 17$)**	**($n = 68$)**	
Testicular volume (mL)	10.3 (8.5;25)	14.2 (9.1;21.6)	0.614
Reduced echogenicity	7 (41.2)	16 (23.5)	0.143
Inhomogeneity	15 (88.2)	63 (92.6)	0.427
Testicular microlithiasis	17 (64.7)	30 (44.1)	0.129
Second affected testis	**($n = 40$)**	**($n = 128$)**	
Testicular volume (mL)	10.4 (8.9;13.3)	16.3 (12.9;19)	<0.001
Reduced echogenicity	14 (35)	16 (12.5)	<0.001
Inhomogeneity	23 (57.5)	18 (14)	<0.001
Testicular microlithiasis	30 (75)	25 (19.5)	<0.001

Note: Values are expressed as median and interquartile ranges and in number and percentage. Comparisons between two groups were performed at each time point using the Mann–Whitney *U* test, Chi-square test, or Fisher's exact test, as appropriate.
Abbreviations: BEP, bleomycin, etoposide, and platinum; BMI, body mass index; RPLND, retroperitoneal lymph node dissection.

studies have already associated increased gonadotropin levels and the presence of oligospermia with the finding of GCNIS in the survival testicle of GCT patients[59–61] and with a higher risk of developing a second tumour.[62,63]

An added value of our work is to reflect testicular dysfunction in coded ultrasound appearance: the mB-GCT group had residual testis with lower testicular volume, more inhomogeneous echotexture, and testicular microlithiasis than U-GCT. The cumulative risk of developing a second tumour for a patient with a reduced and inhomogeneous residual testis is increased, and testicular microlithiasis resulted as an independent risk factor, increasing the chance by more than 30 times.

Evidence suggests that these specific ultrasound characteristics are associated with a greater risk of finding GCNIS in residual testicles during the biopsy, such as low testicular volume,[64–67] inhomogeneous echotexture, and testicular microlithiasis.[65,68] Testicular microlithiasis has been frequently associated with an increase in the development of a testicular tumour, especially in patients with a history of infertility.[69–71] According to a small retrospective study, contralateral testicular cancer was significantly associated with testicular microlithiasis, although no specific risk prediction statistics were performed.[72]

These considerations suggest that ultrasound features of the residual testis may contribute to identifying patients who should undergo closer, but also longer, ultrasound surveillance.

According to EAU guidelines, a contralateral biopsy is suggested in patients with small testicular volume or testicular microlithiasis.[15] This is a safe procedure with no significant contraindications[73] and is considered a valuable tool for detecting GCNIS, even though it is not a routine practice adopted in all countries. The risk of false negatives is low if the procedure is performed at multiple sites and analysed with serial sections and immunohistochemical staining for specific GCNIS

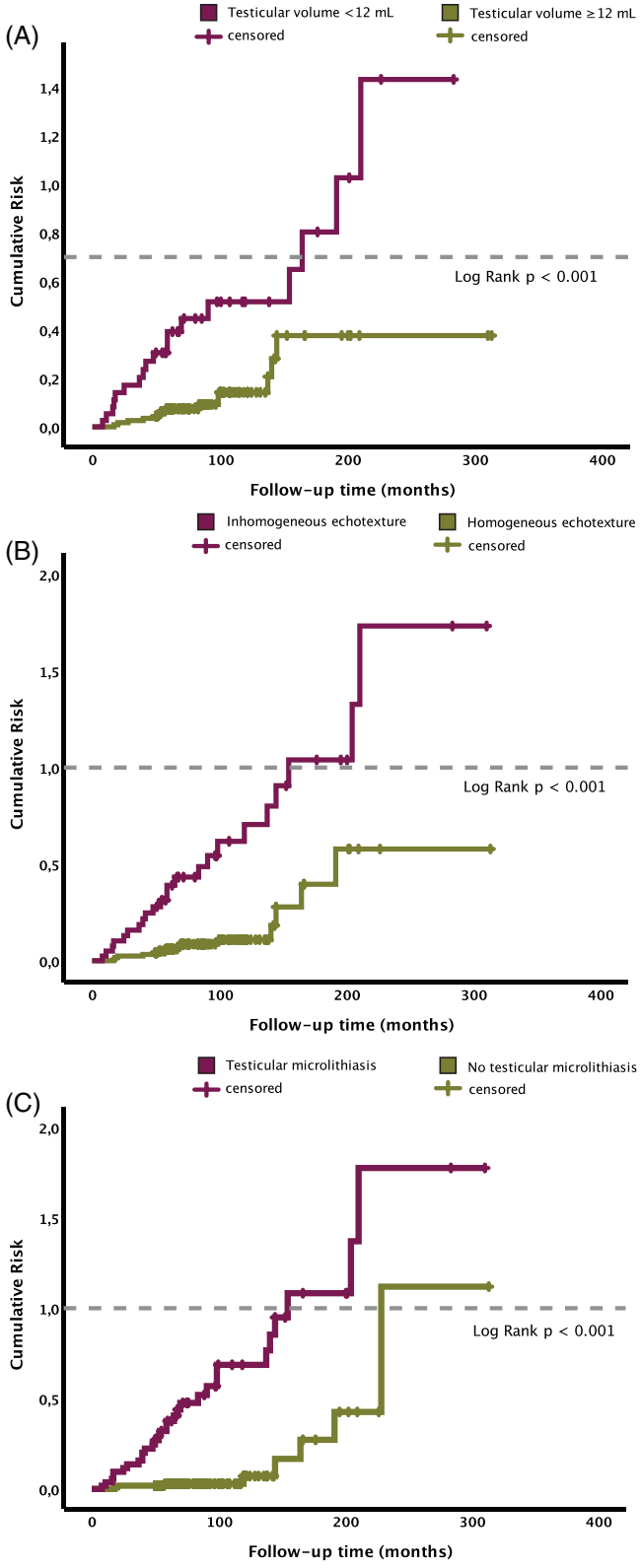

FIGURE 4 Kaplan–Meier curves. The cumulative risk of developing a second tumour increases with low testicular volume(A), inhomogeneous echotexture (B), and testicular microlithiasis(C).

markers by experienced evaluators.[74] Large-scale studies from countries where this procedure is routinely performed have reported the presence of GCNIS in 4–8% of cases, with approximately 50% of these progressing to testicular cancer within 5 years and 70% within 7 years if untreated.[56,67,75,76] In cases of GCNIS, treatment is recommended with low-dose radiotherapy (18–20 Gy) or platinum-based chemotherapy, the latter being less effective but often preferred in patients with advanced stages of GCT.[52,77] Nevertheless, a small number of cases describing new occurrences of GCT or persistence of GCNIS despite radiation treatment are documented in the literature.[52,78]

However, contralateral biopsy followed by additional treatment often poses clinical dilemmas, particularly in young, eugonadal males wishing to father a child, which still represents the majority of patients with the diagnosis of GCT. Radiation treatment, indeed, while preserving Leydig cell function in most cases,[52] unfortunately compromises spermatogenesis.[56,79]

Ultrasound surveillance can offer a viable alternative, particularly in young men aspiring to fatherhood. Avoiding radiation treatment provides patients with extended opportunities for natural or assisted conception, while facilitating early detection of potential testicular malignancies, as supported by our findings. This approach advocates a conservative management strategy that may preclude the necessity for adjuvant treatments. Frequency of US follow-up should be scheduled based on testicular features and the patient's clinical history. Based on our results, US should be performed at least every 6 months in patients with small testes, microlithiasis, and inhomogeneous echotexture for a minimum of 10 years from first cancer, since a specific age threshold of reduced risk of mB-GCT has not been determined.[36,75] Then, self-examination and annual testicular US can be performed, with the duration and the timing of the follow-up adapted based on individual risk factors and based on the patient's age, given that approximately 30% of mB-GCT may appear more than 10 years after the initial diagnosis. In our opinion, long-term US follow-up is crucial even in patients with normal testicular features. This approach, which nonetheless requires high compliance from the patients, ensures a high quality of life for patients with an efficient management of healthcare resources.

The strength of our study is mainly its design. Only patients with regular follow-up and complete clinical data were selected. Furthermore, data on the risk of second cancer are supported by the fact that the control group consists entirely of patients with a follow-up longer than the median time-to-onset of the second tumour and, in any case, greater than 5 years. In addition, exhaustive data collection was performed, including medical history, tumour features, stage, treatment, and ultrasound morphofunctional features of the residual testicle, which is unique in the existing literature. Ultrasound examinations were always performed by the same two operators, experts in testicular pathology, and the images were stored and reviewed by a third expert operator, thus minimising the interoperator variability.[11] Likewise, hormones and semen samples were all analysed in the same laboratory, making data analysis highly reliable.

However, this study has limitations. Firstly, its retrospective design may introduce inherent biases, and the sample size is relatively small compared with other studies, which could impact the generalizability

TABLE 3 Logistic regression with bootstrap analysis ($N = 2000$) predicting the likelihood of developing a metachronous testicular tumour based on functional (total sperm count, LH log values) and morphological (volume, echotexture, and presence of testicular microlithiasis) characteristics of the survival testis.

					95% CI for OR	
	β	SE	p	OR	Lower	Upper
Total sperm count	−0.002	0.005	0.638	0.998	0.988	1.007
LH (log)	1.481	0.971	0.127	4.397	0.656	29.463
Low testicular volume (<12 mL)	0.311	0.787	0.692	1.365	0.292	6.378
Inhomogeneous echotexture	0.652	1.196	0.586	1.919	0.184	19.996
Testicular microlithiasis	3.425	1.129	0.002	30.712	3.357	280.942

Abbreviations: CI, confidence intervals.; OR, odds ration; SE,standard error; β, beta coefficient.

of the findings. Furthermore, there may be potential bias in patient selection, as our institution serves as a reference centre for the early detection and management of testicular neoplasms. This may have contributed to a higher frequency of lower-stage diagnoses. Moreover, as a referral centre for patients from the entire region, individuals often prefer to follow up closer to their homes over an extended period. Consequently, our selected populations may not be directly comparable to registry studies. Finally, while our findings benefit from a decade-long ultrasound experience among the operators, ensuring a high level of standardisation in the ultrasound data collection and interpretation, some sonographic features can be subjectively interpreted, particularly in less specialised centres with lower-quality ultrasound machines, introducing additional biases. However, the attention to high-quality andrological US standardisation is growing steadily in Europe.[22,30,80,81]

5 | CONCLUSION

Ultrasound follow-up is essential for germ cell tumour survivors, given the potential for the late occurrence of second tumours. Features such as reduced testicular volume, inhomogeneous echotexture, and microlithiasis should guide extended follow-up beyond the standard 5-year period.

AUTHOR CONTRIBUTIONS

Marta Tenuta, Carlotta Pozza, and Andrea M. Isidori were involved in conception, design, and coordination. Marta Tenuta, Paola Mazzotta, and Carlotta Pozza performed data acquisition, analysis, interpretation, and drafting. Francesco Angelini, Franz Sesti, Giorgio Franco, Alain J. Gelibter, Iolanda Speranza, and Fabio Massimo Magliocca contributed to data acquisition, patient enrolment, treatment, follow-up, and critical revision. Carlotta Pozza and Andrea M. Isidori performed ultrasound exams; Marta Tenuta reviewed the images. Donatella Paoli and Antonella Anzuini conducted semen and hormone analysis. F.M.M. performed histological examinations. Carlotta Pozza, Daniele Gianfrilli, Francesco Lombardo, Daniele Santini, Giorgio Franco, Enrico Cortesi, Andrea M. Isidori, and Andrea Lenzi were involved in critical revision of the article for important intellectual content and final approval of the version to be published. All authors approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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