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Original Article

Optimized detection of germ cell neoplasia *in situ* in contralateral biopsy reduces the risk of second testis cancer

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Abstract

Objective

To evaluate whether optimized and standardized diagnostic procedures would improve detection of germ cell neoplasia *in situ* (GCNIS) in the contralateral testis of patients with testicular germ cell tumour (TGCT) and decrease the rate of metachronous tumours, which in a nationwide Danish study was estimated to be 1.9%.

Patients and Methods

This was a retrospective analysis of outcomes in 655 patients with TGCT who underwent contralateral biopsies (1996–2007) compared with those in 459 non-biopsied TGCT controls (1984–1988). The biopsies were performed using a standardized procedure with immunohistochemical GCNIS markers and assessed by experienced evaluators. Initial histopathology reports were reviewed, and pathology and survival data were retrieved from national Danish registers. In 604/608 patients diagnosed as GCNIS-negative (four were lost to follow-up), the cumulative incidence of metachronous TGCT was estimated in a competing risk setting using the Grey method. All cases of metachronous TGCT were re-examined using immunohistochemistry.

Results

Germ cell neoplasia *in situ* was found in 47/655 biopsied patients (7.2%, 95% confidence interval [CI] 5.4–9.5%). During the follow-up period (median 17.3 years) five of the 604 GCNIS-negative patients developed a TGCT. In 1/5 false-negative biopsies, GCNIS was found on histological revision using immunohistochemistry and 2/5 biopsies were inadequate because of too small size. The estimated cumulative incidence rate of second tumour after 20 years of follow-up was 0.95% (95% CI 0.10%–1.8%) compared with 2.9% (95% CI 1.3%–4.4%) among the non-biopsied TGCT patients ($P = 0.012$). The estimates should be viewed with caution due to the small number of patients with metachronous TGCT.

Conclusions

Optimized diagnostic procedures improved the detection rate of GCNIS in patients with TGCT and minimized their risk of developing metachronous bilateral cancer. Urologists should be aware of the importance of careful tissue excision (to avoid mechanical compression) and the need of adequate biopsy size. Performing contralateral biopsies is beneficial for patients' care and should be offered as a part of their management.

Keywords

diagnosis, GCNIS, germ cell cancer, immunohistochemistry, testicular biopsy, #TesticularCancer, #tscsm, #uroonc

Introduction

The most common types of testicular germ cell tumours (TGCTs), seminoma and nonseminoma, are derived from germ cell neoplasia *in situ* (GCNIS) [1,2]. Patients with TGCT are at risk of bilateral tumours which may occur synchronously or metachronously in 1.5–5% of cases. The rate of bilateral tumours depends on the population, the study period and the treatment of the primary tumour [3–5]. Incipient neoplasia in the contralateral testis can be detected at the GCNIS stage in approximately 4–6% of patients [6,7]. Patients diagnosed with GCNIS should be treated, with management depending on the patient's situation [8]. In patients with a unilateral TGCT – usually orchiectomized before the contralateral GCNIS is diagnosed – localized low-dose radiotherapy is recommended [8–10]. Chemotherapy is less effective and should not be used in patients with clinical stage 1 disease [9,10]. Active surveillance can be considered for a short period in patients wishing to father a child [8]. In most patients with disseminated TGCT, platinum-based chemotherapy can eradicate GCNIS in the contralateral testis, but approximately 20–30% will still develop metachronous cancer [8,10].

To prevent metachronous second TGCT, screening for GCNIS by performing contralateral testis biopsies has been implemented in Denmark and several centres in other countries (Austria, Germany, the Netherlands). In Denmark, routine screening by means of a single-site surgical biopsy began in 1984 and was implemented nationwide in the late 1980s.

We have previously evaluated the outcome of contralateral biopsies performed nationwide in Denmark between 1984 and 2007 [7]. We found that the cumulative incidence of metachronous TGCT (at a median follow-up of 20 years) was 1.9% among patients who had a GCNIS-negative contralateral biopsy. The difference in this rate compared to the 3.1% observed in a non-biopsied cohort was smaller than we had expected [7]. However, histological revision of biopsies previously judged to be GCNIS-negative from those patients who later developed a metachronous TGCT revealed the presence of GCNIS in a substantial proportion of these cases [7]. In most of these false-negative cases, GCNIS had probably been overlooked because of the lack of immunohistochemical (IHC) staining for a specific marker, which was not generally available for routine use before the mid-1990s. In the nationwide registry study, it was not possible to account for changes in the protocol used for biopsy handling and management of TGCT patients that occurred over the years in different hospitals. Therefore, in this study, we assessed the diagnostic performance and metachronous TGCT-preventive value of contralateral biopsies evaluated only in our specialized centre during a more recent period. Histopathological evaluation was performed according to a standardized protocol, with obligatory IHC staining for GCNIS markers and stringent quality control.

Patients and Methods

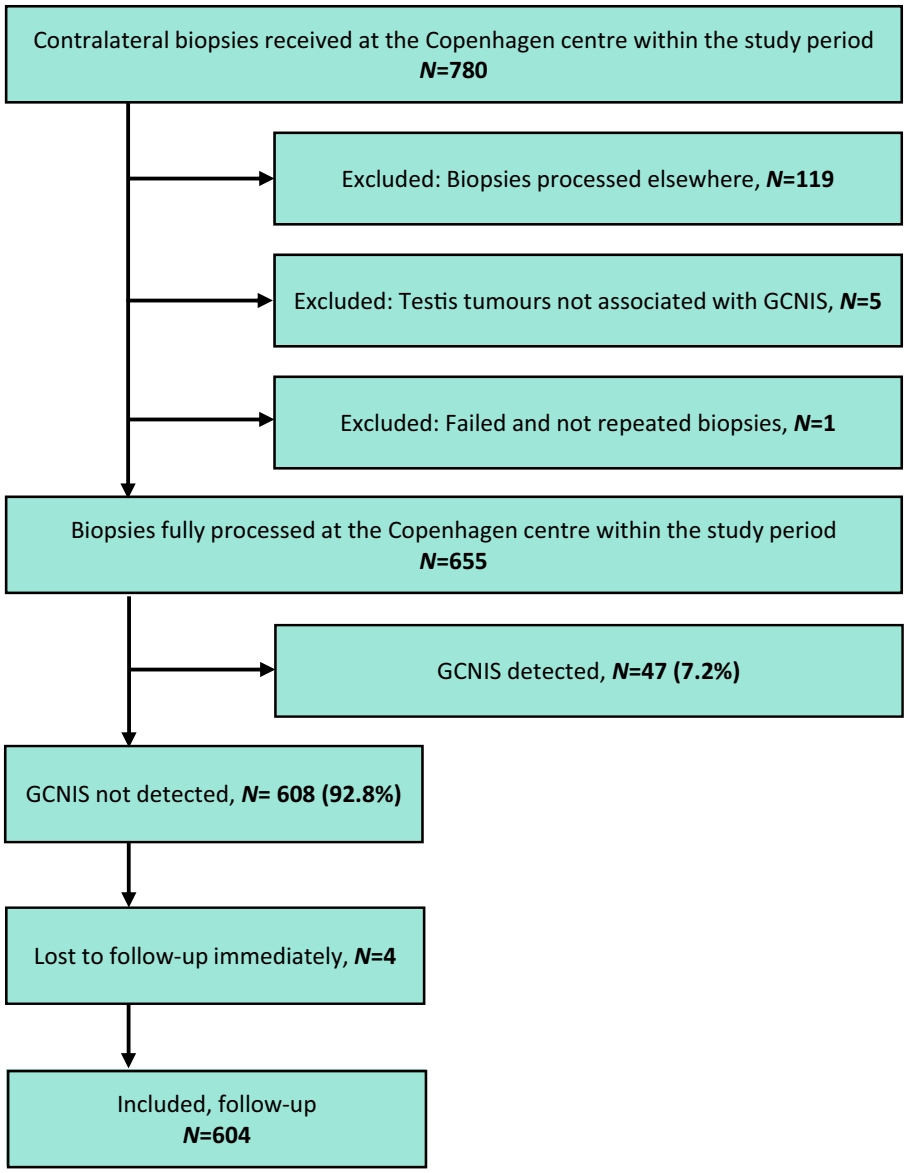
Patients, Inclusion and Exclusion Criteria

Adult patients (N = 780) with testicular cancer (seminomas or nonseminomas), whose contralateral biopsy specimens were evaluated at the Department of Growth and Reproduction, Rigshospitalet, Copenhagen from 30 November 1996 until 31 December 2007, were considered for inclusion this study. These patients were also included in the previous Danish nationwide evaluation of 4130 TGCT patients [7]. The start date was selected because, since November 1996, the standardized protocol of biopsy processing has included obligatory IHC staining and the histological assessment was always performed by two evaluators. The closing date of the end of 2007 was chosen to ensure a sufficiently long follow-up period.

Patients whose biopsy specimens were not fully processed histologically at our department (N = 119) were excluded from the study (Fig. 1). An additional five patients excluded from the study were diagnosed with tumours not derived from GCNIS (Leydig cell tumour or spermatocytic tumour). In 10 patients, the biopsy was not successful because of a surgical or technical failure (epididymis or connective tissue, failed fixation, mechanical traumatization). In 9/10 of these patients, another biopsy was performed and these patients were included in this study, and one patient refused the re-biopsy. In 608 patients, no GCNIS was detected in the contralateral biopsy at the time of primary diagnosis but four patients had to be excluded from the study because of a lack of any follow-up information. Thus, 604 patients were included in the evaluation of the incidence of metachronous TGCTs. Through their personal identification numbers, the included patients were linked to the Danish National Pathology Registry (https://www.patobank.dk/) to retrieve information about their pathological diagnoses, including primary TGCT and later-appearing TGCTs [11]. Information on date of death was retrieved from the Danish Civil Registration System, which also registers causes of death. The patients were followed from the date of their primary TGCT until 31 March 2020, or until they developed a contralateral TGCT, were lost to follow-up, or died, whichever occurred first.

As a control cohort, we included 459 patients diagnosed with TGCT between 1984 and 1988 in hospitals where contralateral biopsies were not yet implemented. Their data were retrieved from the Danish Testicular Cancer (DaTeCa) database [12]. The control group originally included 462 men and was described previously [7]. For the present study, we excluded three patients: one who later had a biopsy performed and received irradiation treatment of GCNIS, one

Fig. 1 Flowchart illustrating the inclusion of patients with contralateral biopsies performed within the study period (30 November 1996–31 December 2007), with exclusion criteria and the numbers of excluded biopsies listed. GCNIS, germ cell neoplasia *in situ*.



who had a synchronous bilateral TGCT and one with a benign testicular tumour (not a TGCT).

Ethical Approval

Retrospective analysis of patient records was approved by the Medical Research Ethics Committee (H-19034972).

Biopsy Procedure, Histological Processing and Evaluation

The contralateral testis biopsies were performed at the time of orchiectomy in 24 hospitals performing urology surgery. The hospitals received vials prefilled with the preferred tissue fixative and detailed instructions from our department, including the recommended biopsy size (minimum size 3 × 3 × 3 mm). The surgical procedure was an open, one-site biopsy through the skin incision, followed by a small incision of the tunica albuginea, and excision of the protruding testis parenchyma. This procedure has a low (<3%) rate of mild complications, is well accepted by patients and is considered safe [13]. We did not systematically collect data concerning side effects of the biopsy, however, we have not seen any serious complications other than manageable bleeding and infections in a small number of patients.

The excised tissue was immersed in the fixative directly at the time of surgery, and the vials were returned to our

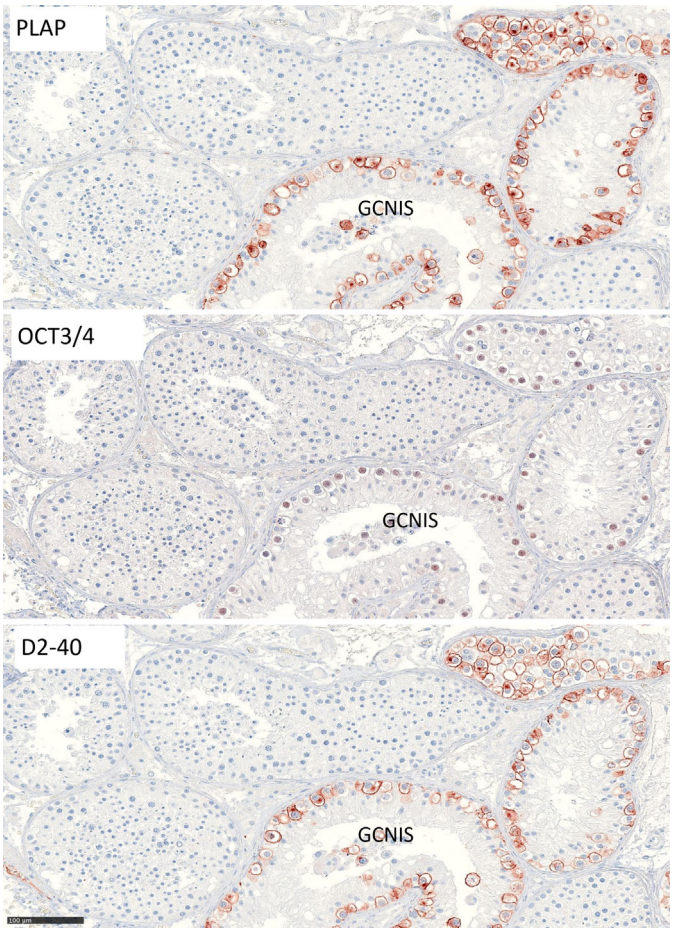
department. Most of the biopsies were fixed in Stieve's fluid [14], but after 2004, we used a less toxic GR fixative, developed in our laboratory (200 mL 37% formaldehyde and 40 mL 100% acetic acid mixed with 760 mL PBS buffer 50 mmol/L, pH 7.4). After overnight fixation, the tissues were dehydrated, cleared in xylene and embedded in paraffin. The blocks were then cut into approximately 80–100 serial sections (depending on the specimen size), which were divided among 12 glass microscopy slides: six slides for staining with haematoxylin and eosin, two slides for periodic acid–Schiff, and four slides for IHC staining. Additional sections were cut and reserved for post-analysis IHC testing if needed. Antibodies for routine IHC staining included one or two different markers for GCNIS (and most types of malignant germ cell tumours): placental-like alkaline phosphatase (PLAP) and D2-40 antigen (M2A) which in the testis tissue recognizes (**podoplanin** [PDPN]) and is present in GCNIS and lymphatic epithelium. Both antibodies were from DAKO (Glostrup, Denmark) [15,16], and were selected because of their robust performance in the special fixatives used. Since 2004, an antibody recognizing an octamer-binding transcription factor (OCT)3/4 (Santa Cruz Biotech, Santa Cruz, CA, USA) [17] was also used for selected biopsies where re-evaluation was performed. These markers were investigated in separate studies, and their high specificity for GCNIS was confirmed [15–17]. IHC staining was performed using an indirect peroxidase method, as previously described [15–17]. Each IHC staining run included positive (sections with GCNIS) and negative controls (primary antibodies replaced by dilution buffer). Typical examples of stained biopsies are shown in Fig. 2.

The stained sections were examined independently by two experienced evaluators (N.E.S. and E.R.M.) using a light microscope. There were no discrepancies with regard to presence of GCNIS. Any other discrepancies in the evaluation (e.g., concerning Leydig cell clustering or presence of immune infiltrates) were discussed and consensus was reached. The histopathology report always included a note on presence or absence of GCNIS, microlithiasis, inflammatory cells, invasive spread of tumour cells, and semiquantitative evaluation of spermatogenesis, according to our standard protocol [18]. Each report included a note on the size and technical quality of the biopsy. Among the biopsies included in this study, 5.3% were described by the evaluators as technically inadequate, mainly because they were too small or because of mechanical tissue disruption. The reports were stored in a laboratory database. All mounted sections and tissue blocks were stored in our department's tissue archive.

Re-evaluation of Biopsies in Patients with Metachronous TGCT

In patients who developed metachronous cancer within the follow-up period, the initial reports and the archived sections were re-examined. Any remaining tissue was sectioned and IHC-stained again for at least two different markers, PLAP, D2-40 or OCT3/4, as described above. The revision was performed by two independent evaluators using a light microscope or slide scanning microscopy (NanoZoomer 2.0 HT; Hamamatsu Photonics, Herrsching, Germany).

Fig. 2 Serial sections of the contralateral testis biopsy positive for germ cell neoplasia *in situ* (GCNIS) from a patient with unilateral testicular germ cell tumour. The biopsy shows immunohistochemical expression of placental-like alkaline phosphatase (PLAP), OCT3/4 factor and D2-40 antigen, marking GCNIS cells in several tubules. Note that PLAP and D2-40 are present in cell membranes, whereas OCT4 is a nuclear marker. Scale bar equals 100 μm.



Statistical Analysis

The patient data from the laboratory database and register databases were merged into a separate database. Estimation of the proportion of metachronous cancer in the screened vs the unscreened cohort that appeared over time after diagnosis of the first TGCT and the contralateral biopsy (cumulative incidence) was carried out in the case and control cohorts. This estimation was performed according to the Grey method

using the '*cmprsk*' package in R software [19]. Using this approach, death is considered as a competing risk, and the resulting incidence is the probability that a patient will experience a metachronous cancer as a function of follow-up time from the biopsy. Patients were censored at the end of the study period on 31 March 2020, or when they developed a contralateral TGCT, died, or were lost to follow-up. Results were similar when using age as the underlying time scale and patients' ages at biopsy were treated as a delayed entry (data not shown). Descriptive statistics were performed using IBM SPSS Statistics software.

Results

Germ cell neoplasia *in situ* in the contralateral testis was detected at the initial investigation in 47/655 patients (7.2%, 95% CI 5.4–9.5% [Fig. 1]). The patients with GCNIS were slightly younger (median 31.0 years) than those without GCNIS (median 34.0 years; $P = 0.04$). A summary of the data in the screened and unscreened patients is presented in Table 1.

Metachronous TGCT was later diagnosed in five of the 604 screened patients with complete follow-up data (details in Table 2). Biopsies of three of these five patients were judged to be of good technical quality, and in one of these three, GCNIS was detected in additional sections cut from the archived block and IHC-stained for GCNIS markers (Table 2; Patient 1). The remaining two biopsies were described in the initial report as sub-optimal, due to their small size, and GCNIS was not detected in the archived tissue remnants (Table 2; Patients 2 and 4).

Among the 604 GCNIS-negative patients, the estimated cumulative incidence of metachronous tumours after 20 years was 0.95% (95% CI 0.10–1.8%). In the unscreened cohort of 459 TGCT patients, the cumulative incidence of metachronous TGCT was 2.9% (95% CI 1.3–4.4%) after 20 years of follow-up, and 3.3% (95% CI 1.7–4.9%) after 30 years from the initial diagnosis. Figure 3 shows the lower probability of developing a metachronous TGCT in the screened vs unscreened cohort ($P = 0.012$) as well as the results of a sub-analysis performed after excluding the 41 patients with biopsies described as technically inadequate ($P = 0.003$).

Discussion

We examined the value of contralateral testicular biopsy as a tool for detection of GCNIS to minimize the risk of the development of metachronous TGCT in a cohort of TGCT patients evaluated in our laboratory after implementation of an optimized and currently used procedure. We observed an estimated cumulative 20-year incidence rate of 0.95% for metachronous TGCT in the patients evaluated in the present study. This was lower than the 1.9% reported in the previous Danish nationwide population study [7] that included biopsy specimens evaluated mainly by conventional histological methods at multiple hospitals.

Our centre in Copenhagen was the first to implement contralateral biopsies to screen for GCNIS as a routine procedure [20]. GCNIS detection was initially based on morphological recognition alone and required substantial experience on the part of the evaluator. Detection sensitivity has improved significantly since the implementation of serial sections and IHC staining for specific markers of GCNIS, especially if two different markers are used [15,18,21,22]. It should be emphasized that the specificity of the commonly

Table 1 Summary of clinical characteristics and findings in two cohorts of testicular cancer patients: those with contralateral testicular biopsies evaluated at a specialized centre and the non-screened controls.

	Cohort with contralateral biopsies (1996–2007)			Unscreened cohort (1984–1988)
	Total, *N* = 655	GCNIS +, *N* = 47	No GCNIS, *N* = 608	Total, *N* = 459
Age at primary diagnosis, years	33.7 (21.6–55.5)	31.0 (19.2–50.2)	34.0 (21.8–56.2)	34.4 (20.7–58.9)
Histology (primary TC)				
Seminoma, %	55.3	63.8	54.6	60.3
Nonseminoma, %	44.7	36.2	45.4	39.7
Treatment (primary TC)				
Orchiectomy alone, %	53.5	48.9	53.7	51.9
Additional chemotherapy, %	32.2	31.9	32.2	26.6
Additional radiotherapy, %	14.4	19.1	14.0	21.4
Contralateral GCNIS*, %	7.2	100	0	n.a.
Biopsies technically adequate, %	94.7	97.9	94.4	n.a.
Follow-up time, years	17.4 (7.1–22.8)	18.4 (6.5–23.2)	17.3 (9.9–22.8)	25.5 (4.3–28.4)
Lost to follow-up, %	0.6	0	0.7	1.7
Died, %	8.5	8.5	8.6	22.0
Metachronous TC, *n* (%)	7 (1.1)	2 (4.3)*	5 (0.8)	15 (3.2)
Estimated cumulative rate after 20 years)	n.a.	n.a.	0.95%	2.9%

*GCNIS, germ cell neoplasia in situ; n.a., not applicable or not assessed; TC, testicular cancer. Results are shown as medians (5–95 percentiles) unless otherwise stated. *All GCNIS patients treated with radiotherapy, 2/47 insufficiently treated with 14 Gy and developed a metachronous TC.*

Table 2 Description of the patients (all testicular germ cell tumour clinical stage 1), who developed a metachronous cancer despite negative results of the screening for germ cell neoplasia *in situ* in the initial contralateral biopsy.

Patient no.	Age at primary tumour, years	Primary tumour (side)	Contralateral biopsy description, incl. technical quality and size	Age at second tumour, years	Interval between tumours, years	Second tumour (side)	Revision of the primary biopsy
1	47	Seminoma (L)	No GCNIS (PLAP-negative), preserved spermatogenesis, some inflammatory cell infiltration. Good technical quality, a large biopsy.	54	7	Seminoma (R)	GCNIS found in the remaining tissue fragment (positive for PLAP and D2-40)
2	37	Seminoma (R)	No GCNIS (PLAP-negative), preserved spermatogenesis, but some tubules with spermatogenic arrest. A small and traumatized biopsy.	51	14	Seminoma (L)	Same pattern as in the original description, no GCNIS
3	29	Seminoma (L)	No GCNIS (PLAP-negative, D2-40-negative), decreased spermatogenesis, moderate inflammatory cell infiltration. Good technical quality.	35	6	Seminoma (R)	Same pattern as in the original description, no GCNIS
4	23	Seminoma (R)	No GCNIS (PLAP-negative), preserved spermatogenesis. A small biopsy, size $1 \times 1 \times 1$ mm.	33	10	Seminoma (L)	Same pattern as in the original description, no GCNIS
5	40	Seminoma (L)	No GCNIS (PLAP-negative, D2-40-negative), preserved spermatogenesis. Good technical quality and size (>40 tubules visible).	57	16	Seminoma (R)	Same pattern as in the original description, no GCNIS

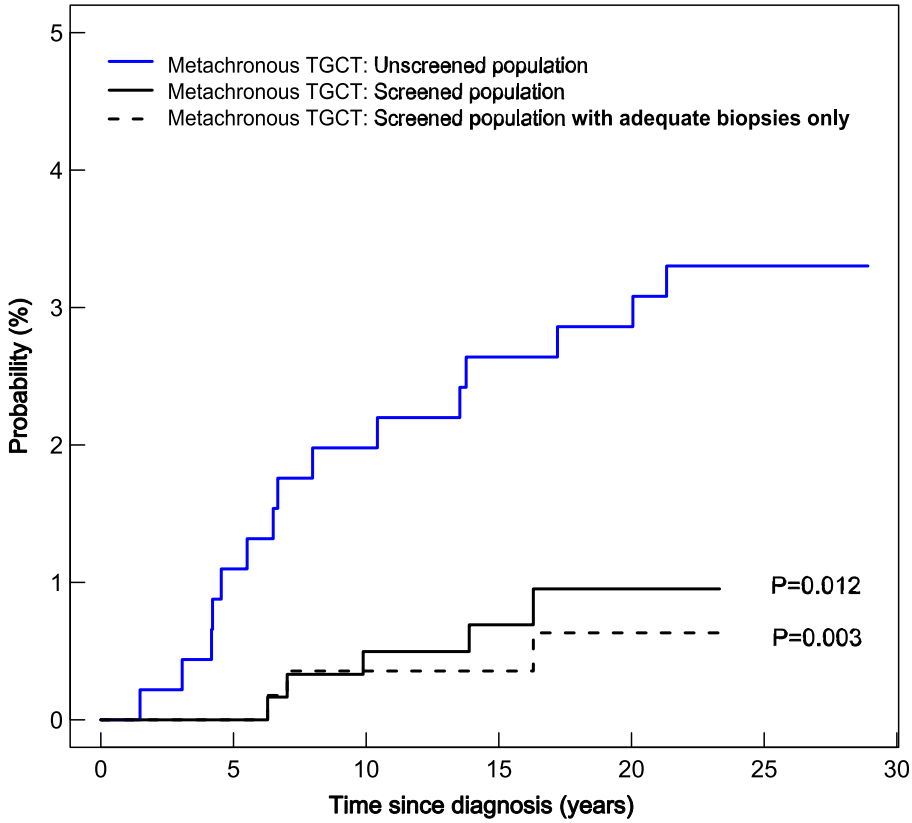
GCNIS, germ cell neoplasia in situ; PLAP, placental-like alkaline phosphatase.

used GCNIS markers is very high because these proteins/antigens are not normally present in the adult germ cells, and the available antibodies are robust and validated by numerous studies. Hence, every pathology department can now carry out the analysis without the risk of either overlooking GCNIS or obtaining a false-positive result, which could potentially lead to overtreatment of the patient.

In the present study, GCNIS in the contralateral testis was detected in 7.2% of the patients, which is a higher rate than in other comparatively large studies, which have reported a GCNIS rate of approximately 5% [6]. This rate is also higher than the 4.4% observed in the previous nationwide Danish study [7]. The selection of patients referred to our tertiary centre could be a confounding factor. The possibility of increasing prevalence of contralateral GCNIS in more recent decades could also be considered and would be consistent with the reported change in the prevalence of metachronous bilateral TGCT, which has risen within a couple of decades from 1.9% to 3.8% in Norway [3] and from 1.7% to 3.8% in the cohort from Memorial Sloan Kettering Cancer Center in the USA [23]. We believe, however, that our optimized protocol for biopsy evaluation most likely contributed to the greater rate of GCNIS detection in the present study. In the previous study, we found unrecognized GCNIS upon revision in one-third of biopsies reported as GCNIS-negative in patients who developed metachronous TGCT [7]. In a large study from Germany, the rate of false-negative biopsies was 0.5% and in five of 21 available specimens, GCNIS was found at re-examination [21]. Similarly, in a study from the Netherlands, GCNIS or micro-invasive tumours were found upon revision in approximately half of the 'negative' biopsies performed in infertile patients who later developed TGCT [22].

In our experience, failure to detect GCNIS in testis biopsies is often caused by substandard technical quality. Technical issues are important, especially adequate biopsy size and careful handling by the surgeon excising the biopsy [21]. In our series, 5.3% of biopsies were technically suboptimal because they were too small or because of mechanical trauma, including two apparently false-negative biopsies in patients with metachronous TGCT. The chance of missing GCNIS could potentially be diminished by taking additional tissue samples from the contralateral testicle, and this has been evaluated previously. Two-site biopsies were judged to significantly increase diagnostic accuracy, and some centres have even performed three-site biopsies [6]. We believe that a single biopsy is sufficient to rule out the occurrence of GCNIS in the majority of patients. However, based on the results of this and previous studies, an elective two-site biopsy can be considered in some patients, especially in

Fig. 3 Probability of developing a metachronous contralateral testicular germ cell tumour (TGCT) in the screened vs unscreened cohort. Death was treated as a competing risk. The result for the entire group of 604 patients with germ cell neoplasia *in situ*-negative biopsies is shown as 'Screened population'. The result in the screened cohort excluding the 5.6% of patients whose testicular contralateral biopsies had been described as technically inadequate is shown as 'Screened population with adequate biopsies only'.



younger men with normal-sized testicles (15–18 mL), who may benefit from extended biopsy material [6,21,24]. Contralateral GCNIS in men with testis volume > 18 mL, however, is rare [24]. The risk of contralateral GCNIS or metachronous bilateral cancer was reported to decrease with age [6,7,24,25] but a specific age threshold has not been determined.

We believe that developing non-invasive and more sensitive methods for the detection of GCNIS would be optimal. However, efforts have been disappointing so far; the sensitivity of the immunocytological method of detection of GCNIS cells in ejaculates is too low [26], nor is it yet possible to use micro-RNA based assays (e.g., miR-371a-3p, a promising serum marker for overt TGCT) for detection of GCNIS in serum or seminal fluid because of low sensitivity and the presence of miR-371a-3p in non-malignant conditions [27].

Theoretically, a well-functioning screening programme for GCNIS should eliminate the development of invasive cancer in the contralateral testicle. Although not zero, the cumulative incidence rates of metachronous TGCT of 1.9% in the previous nationwide Danish study [7] and the reduced rate of 0.95% found in the present study, based exclusively on optimized methodology, show that screening for GCNIS can eliminate a large proportion of metachronous invasive tumours. In countries where contralateral biopsies are not routinely performed, the reported incidence of metachronous TGCT (after a similar follow-up period) is higher: approximately 3.4%–4.0% in cohorts from Norway [3], the USA [23], the Netherlands [25] and Spain [28].

This study has some limitations, including the limited re-evaluation, which was performed only in the biopsy material from patients who developed a metachronous TGCT. Another limitation is the relatively small cohort; given that a second TGCT after a GCNIS-negative biopsy is a rare event, a few cases can have a considerable effect on the metachronous cancer rate and the *P* value. The difference in the follow-up length between the biopsied cohort and the control cohort is also a limitation, although we have reduced this bias by estimation of the cumulative rates. The length of the follow-up is important because a metachronous TGCT in the contralateral testis can appear as late as 14–28 years after the primary tumour [7,25,29] and such long intervals were

also observed in two patients in the present study. Finally, the control cohort was diagnosed and treated approximately a decade previously, when treatment protocols were different to some extent. Even though the proportions of patients treated by surgery and surveillance alone or with chemotherapy were comparable, the chemotherapy regimens were adjusted over the years, and the use of adjuvant radiotherapy decreased with time (Table 1).

It is important to emphasize that platinum-based chemotherapy regimens implemented in the more recent period have undoubtedly eradicated undetected GCNIS in some patients, as shown by several studies [7,25,28,30,31]. For example, in a recent Dutch study, the rates of metachronous TGCT were 4.4% and 1.7% in patients not treated vs treated with platinum-based regimens, respectively [25]. On the other hand, studies have shown that chemotherapy is not efficient for treating GCNIS alone [7,10], and that approximately one-third of patients treated with chemotherapy for the primary TGCT would develop a contralateral tumour [5,25,28,30,31]. Furthermore, current management guidelines increasingly favour surveillance without adjuvant therapy for TGCT patients in clinical stage I [30,32], but patients on surveillance have an increased risk of metachronous TGCT [31] and require intensive follow-up of the contralateral testis, which can be eased in patients with a GCNIS-negative contralateral biopsy [32].

Radiotherapy is effective in preventing TGCT in patients harbouring GCNIS [9,10]. In our cohort, 47 patients were correctly diagnosed with GCNIS at the initial investigation but two of these patients, treated with an insufficient dose (14 Gy) of radiotherapy, later developed a metachronous TGCT. However, of the 45 patients (96%) who received the current standard irradiation treatment (2Gy x 8 at our centre), none has developed a metachronous TGCT. Thus, the appearance of second testis cancer, which would require renewed treatment and an extended follow-up period, was prevented in up to 7% of the patients in our cohort. Hence, the use of screening for contralateral malignancy, which is currently not universally recommended, ought to be reconsidered, at least in younger patients, who, according to the present study have a greater probability of harbouring GCNIS in the contralateral testis, and who can derive the greatest benefit from the simultaneous evaluation of spermatogenesis. Conversely, the risk of metachronous TGCT decreases in older men, but a clear point of intersection has yet to be defined. In patients with fertility concerns, radiotherapy of GCNIS can be postponed until after cryopreservation of sperm or natural paternity has been secured. A discussion of the pros and cons with each patient is important and factors such as the patient's age, testicular size, semen quality, the presence of risk factors, fertility concerns and personal priorities should be taken into consideration.

In conclusion, in this cohort, the patients with testicular cancer who had a GCNIS-negative contralateral testis biopsy had a significantly lower risk of a metachronous invasive germ cell tumour than the non-biopsied patients. The standardized and optimized biopsy protocol with a panel of IHC GCNIS markers has substantially improved recognition of GCNIS. Urologists need to be aware of the importance of the technical quality of surgical procedure, especially gentle tissue handling and an adequate biopsy size. In our opinion, performing contralateral biopsies is beneficial for patients' care and should be offered as a part of their management.

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Disclosure of Interests

None of the authors has any conflict of interest to declare.

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Abbreviations: GCNIS, germ cell neoplasia *in situ*; IHC, immunohistochemistry; OCT3/4, octamer-binding transcription factor 3/4; PLAP, placental-like alkaline phosphatase; TGCT, testicular germ cell tumour.