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Testicular cancer survivors in the cisplatin era: Metachronous contralateral testicular cancer, second cancer and causes of death

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Abstract

Background/aims: Testicular cancer (TC) is the most common cancer among young men aged 20-40 years. The cure rates are excellent, an important factor being the chemotherapeutic agent cisplatin. This thesis aimed to investigate how TC treatment influenced the subsequent risk for metachronous contralateral (second) TC, non-TC second cancer (SC) and non-TC mortality.

Methods: The Cancer Registry of Norway (CRN) identified all men diagnosed with TC 1980-2009. Complete TC treatment information was retrieved from medical journals for all eligible men (n=5724), and linked with the CRN and the Norwegian Cause of Death Registry. Crude cumulative incidences were estimated, and standardized incidence ratios (SIRs) and standardized mortality ratios (SMRs) were calculated to compare rates with the general population. Adjusted hazard ratios (HRs) were estimated to investigate the effect of treatment intensity.

Results: Median follow-up time in the three papers was 16.6-18.7 years. Second TC developed in 218 (3.9%) men, and the 20-year cumulative incidence was 4% (95% CI 3.5-4.6). Treatment with cisplatin-based chemotherapy (CBCT) at first TC was associated with a significantly reduced second TC risk compared with surgery (HR 0.55). A dose-dependent relationship was observed, with a risk reduction for each additional CBCT cycle after 3, 4 and >4 cycles (HRs 0.53, 0.41 and 0.21, respectively). Additionally, older age at first TC was associated with a reduced second TC risk. Overall, 572 (10.2%) men developed a non-TC SC, and compared with the general population the risk was increased after treatment with surgery (SIR 1.28, 95% CI 1.05-1.56), CBCT (SIR 1.62, 95% CI 1.39-1.88) and radiotherapy (SIR 1.64, 95% CI 1.46-1.85). In total, 665 (12%) men died due to non-TC causes during follow-up, and the risk was increased after CBCT (SMR 1.23, 95% CI 1.07-1.43) and radiotherapy (SMR 1.28, 95% CI 1.15-1.43), but not after surgery. Compared with the general population, increased risk for suicide was observed after treatment with CBCT. The highest risk for SC and non-TC mortality was observed in those with young age at TC diagnosis. Compared with surgery, treatment with 1 CBCT cycle was not associated with increased risks, while increased risks were observed after ≥2 (SC) and ≥3 (mortality) CBCT cycles in those with >10 years follow-up.

Conclusions: Previous TC treatment as well as age at diagnosis influenced the subsequent risks for second TC, SC and premature non-TC mortality. This information is important for all TC survivors and for health personnel involved in the follow-up.

Sammendrag

Bakgrunn: Testikkelkreft er den vanligste kreftsykdommen blant unge menn. Heldigvis kureres de aller fleste, og cellegiften cisplatin er en viktig årsak til dette. Målsetninga med denne avhandlinga var å undersøke hvordan testikkelkreftbehandling påvirker den seinere risiko for å utvikle metakron kontralateral testikkelkreft (ny testikkelkreft), sekundærkreft (ikke testikkelkreft) og risiko for død av andre årsaker enn testikkelkreft.

Metode: Kreftregisteret identifiserte alle menn diagnostisert med testikkelkreft i perioden 1980-2009. Komplett informasjon om behandling ble samla fra medisinske journaler for alle menn inkludert i studien (n=5724) og koblet med data fra Kreftregisteret og Dødsårsaksregisteret. Kumulativ insidens ble estimert. For å sammenligne med den generelle befolkning kalkulerte vi standardisert insidensratio (SIR) og standardisert mortalitetsratio (SMR). Justerte hasard ratio (HR) ble estimert for å undersøke effekten av behandlingsintensitet.

Resultater: Median oppfølgingstid i de tre arbeidene var 16.6-18.7 år. Det var 218 (3.9%) menn som utviklet en ny testikkelkreft, og 20 års kumulativ insidens var 4% (95% konfidensintervall (KI) 3.5-4.6). Behandling med cisplatin-basert cellegift (CBCT) ved første testikkelkreft var assosiert med en signifikant reduksjon i risiko for ny testikkelkreft sammenligna med kirurgi (HR 0.55). Vi observerte en dose-respons-sammenheng med en risikoreduksjon for hver påfølgende kur med CBCT etter 3 (HR 0.53), 4 (HR 0.41), >4 (HR 0.21). Alder >30 år ved første testikkelkreft var også assosiert med redusert risiko for en ny testikkelkreft. Totalt var det 572 (10.2%) menn som utviklet sekundærkreft, og sammenligna med den generelle befolkning var risikoen økt etter kirurgi (SIR 1.28, 95% KI 1.05-1.56), CBCT (SIR 1.62, 95% KI 1.39-1.88) og strålebehandling (SIR 1.64, 95% KI 1.46-1.85). Det var 665 (12%) menn som døde av andre årsaker enn testikkelkreft, og risikoen var forhøyet etter behandling med CBCT (SMR 1.23, 95% KI 1.07-1.43) og strålebehandling (SMR 1.28, 95% KI 1.15-1.43), men ikke etter kun kirurgi. Sammenligna med generell befolkning fant vi en økt risiko for selvmord etter behandling med CBCT. Den høyeste risikoen for sekundærkreft og død (ikke testikkelkreft) ble observert blant de som var yngst ved testikkelkreftdiagnosen.

Konklusjon: Testikkelkreftbehandling og alder ved diagnose påvirker den seinere risikoen for ny testikkelkreft, sekundærkreft og for tidlig død. Dette er viktig informasjon for testikkelkreft-overlevere og helsepersonell involvert i oppfølginga av denne gruppa kreftoverlevere.

Disclaimer

The study has used data from the Cancer Registry of Norway. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway is intended nor should be inferred.

List of papers

The thesis is based on the following papers:

I **Continuing increased risk of second cancer in long-term testicular cancer survivors after treatment in the cisplatin era**

R Hellesnes, O Kvammen, TA Myklebust, RM Bremnes, A Karlsdottir, HFS Negaard, T Tandstad, T Wilsgaard, SD Fossa and HS Haugnes.

International Journal of Cancer, 147:21-32, 2020

II **Metachronous contralateral testicular cancer in the cisplatin era: a population-based cohort study**

R Hellesnes, TA Myklebust, RM Bremnes, A Karlsdottir, O Kvammen, HFS Negaard, T Tandstad, T Wilsgaard, SD Fossa and HS Haugnes.

Journal of Clinical Oncology, 39:308-318, 2021

III **Testicular cancer in the cisplatin era: Causes of death and mortality rates in a population-based cohort**

R Hellesnes, TA Myklebust, SD Fossa, RM Bremnes, A Karlsdottir, O Kvammen, T Tandstad, T Wilsgaard, HFS Negaard, and HS Haugnes.

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Abbreviations

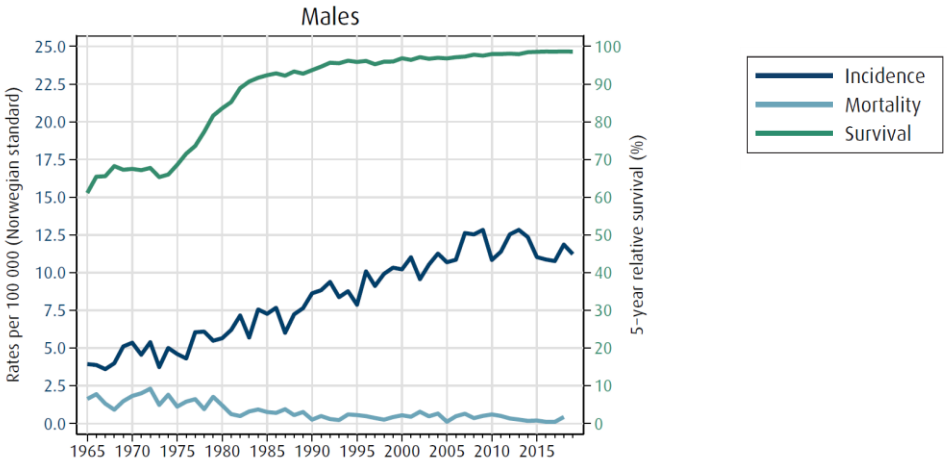
AML	Acute myeloid leukemia
BEP	Bleomycin, etoposide and cisplatin
CBCT	Cisplatin-based chemotherapy
CI	Confidence interval
CRN	The Cancer Registry of Norway
CVB	Cisplatin, vinblastine and bleomycin
CVD	Cardiovascular disease
EP	Etoposide and etoposide
FSH	Follicle-stimulating hormone
GCNIS	Germ cell neoplasia in situ
Gy	Gray
HR	Hazard ratio
ICD	International Classification of Diseases
LH	Luteinizing hormone
MDS	Myelodysplastic syndrome
NCoDR	The Norwegian Cause of Death Registry
PBCT	Platinum-based chemotherapy
RPLND	Retroperitoneal lymph node dissection
RT	Radiotherapy
SC	Second cancer (excluding testicular cancer)
SEER	Surveillance, Epidemiology and End Results
SIR	Standardized incidence ratios
SMR	Standardized mortality ratios
SNPs	Single nucleotide polymorphisms
SWENOTECA	The Swedish and Norwegian Testicular Cancer Group
TC	Testicular cancer
TCS	Testicular cancer survivors
TDS	Testicular dysgenesis syndrome
WHO	The World Health Organization

1 Introduction

1.1 Background and epidemiology of germ cell testicular cancer (TC)

Germ cell testicular cancer (TC) is a rare malignancy as it represents only 1-2% of all malignancies in Norway, corresponding to about 300 new TC cases annually. Despite this, it is the most common cancer in men aged 15-49 years in Norway.[1] Without a clear explanation, the TC incidence in Norway increased gradually from 1965, with a plateau reached in 2007 with an age-adjusted (world standard) incidence rate of 12.8/100.000 (Figure 1).[1,2] In 2019, the incidence rate was 10.7/100.000.[1] Europe has the highest incidence of TC in the world, and the incidence has been increasing since at least mid-20th century.[3] Northern European countries, with Norway and Denmark as the countries with the highest incidences, have had the topmost incidence rates, but it has stabilized somewhat in recent years. Both the rising incidence and the stabilization are largely unexplained.[3] During the last decades, a rising incidence has been observed in Eastern and Southern Europe, and the incidence has also been increasing in some Middle Eastern countries as well as countries in Latin America and the Caribbean.[3]

Figure 1. Age-adjusted incidence, mortality and 5-year relative survival of testicular cancer in Norway from 1965-2019.[1] The Cancer Registry of Norway, Cancer in Norway 2019

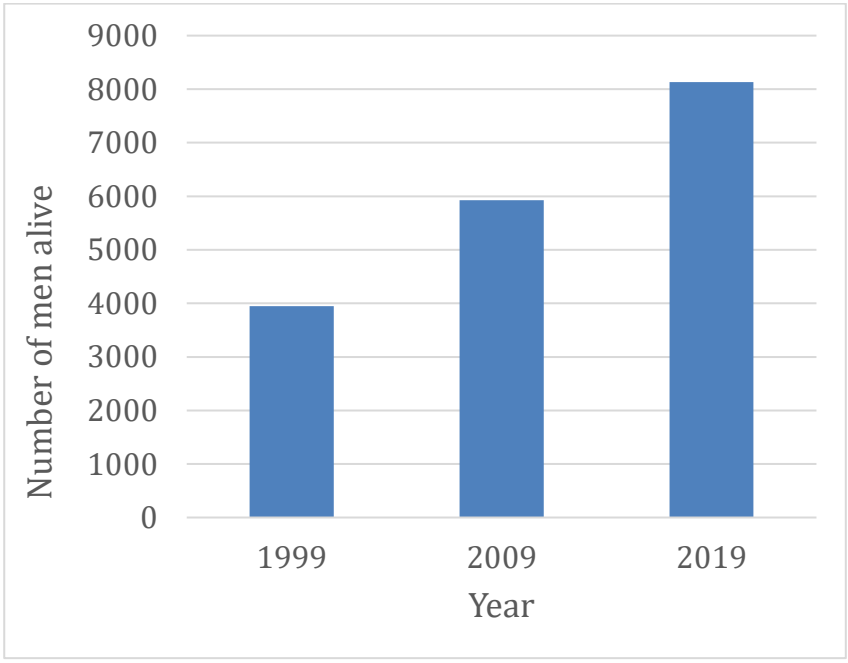


Fortunately, the survival rates are excellent with 15-year relative survival rates exceeding 98% in Norway (Figure 1), and TC now has the highest five-year relative survival among all cancers in Norway.[1] In Europe, the age-standardized 5-year relative survival has been around 90% from 1999-2007, and the 5-year relative survival conditional to surviving one year is 95% in most countries.[4] However, mortality rates vary across different countries.[5]

As demonstrated in Figure 1, the survival improved dramatically during the late 1970s, the most important reason being the introduction of cisplatin in the treatment of metastatic TC.[6] Additionally, treatment according to a risk stratification system with serum tumor markers elevation and half-life to guide diagnosis and treatment effect, improvements of diagnostic imaging, timing of surgery of residual masses, potent salvage chemotherapy and multimodal therapy are important co-factors for the high cure rates.[7-10]

As a consequence of the increased incidence and the exceptionally high survival, the number of long-term TC survivors (TCS) has been growing. The number of Norwegian TCS alive more than 10 years after diagnosis have increased from 3400 in 2009 to 5221 in 2019.[1,11] Likewise, the prevalence of men alive in Norway with a history of TC has doubled during the last two decades as demonstrated in Figure 2.

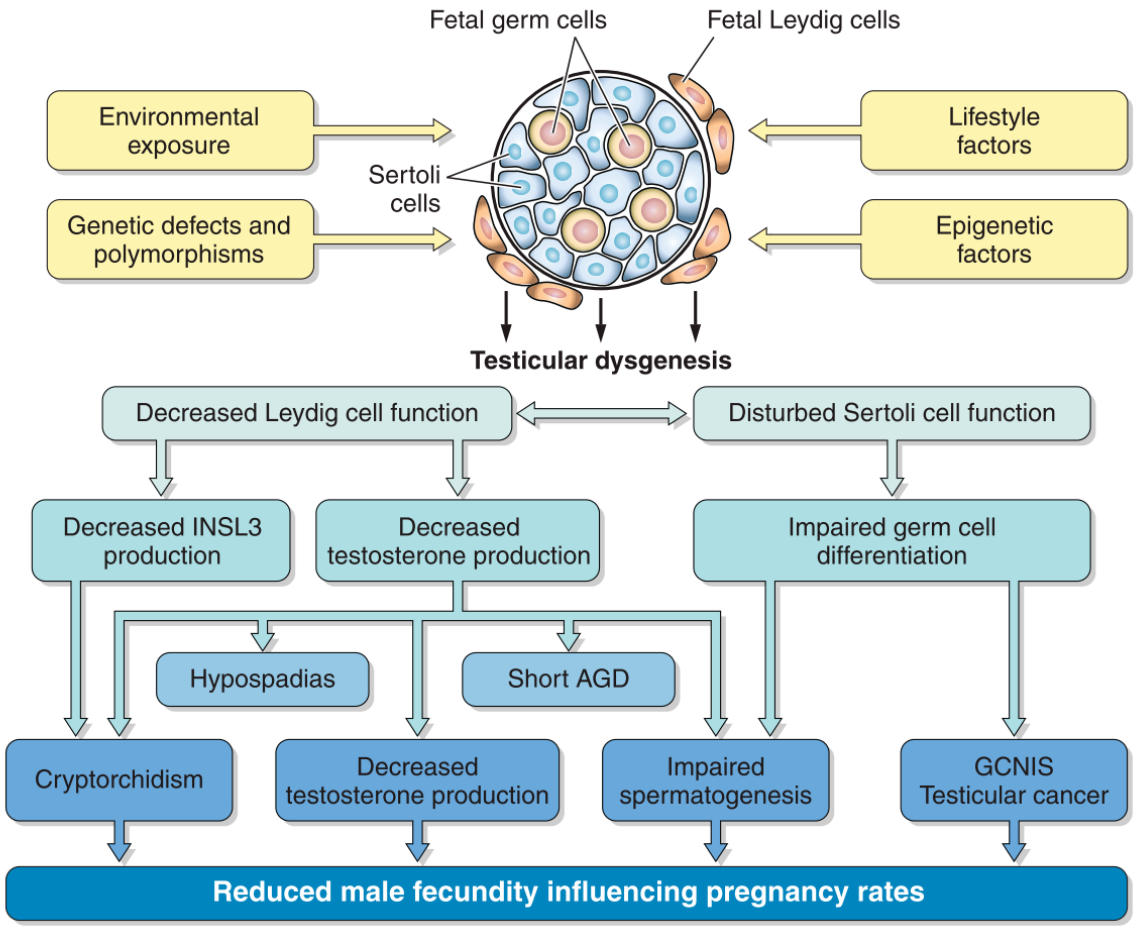
Figure 2. Number of men alive in Norway with a history of testicular cancer diagnosis in 1999, 2009 and 2019. Numbers from the Cancer Registry of Norway.[1,11]



1.2 Risk factors and pathogenesis of germ cell TC

The two main functions of the testicles are the sperm cell production (by Sertoli cells) and the testosterone production (by Leydig cells).[12] Several traits are associated with TC; atrophic testis (<12 mL), cryptorchidism, hypospadias, microlithiasis, infertility or low sperm count, as well as a family history of TC.[13-15] These features often co-exist in the same individuals, and the associations between them led to the hypothesis of a testicular dysgenesis syndrome (TDS), which can be thought of as a form of sex development disorder, with TC as the most serious condition (Figure 3).[13,14]

Figure 3. The hypothesis of the testicular dysgenesis syndrome and signs that might be linked to it.[13] Permission obtained from The American Physiological Society.



Abbreviations: AGD, ano-genital distance; GCNIS, germ cell neoplasia in situ

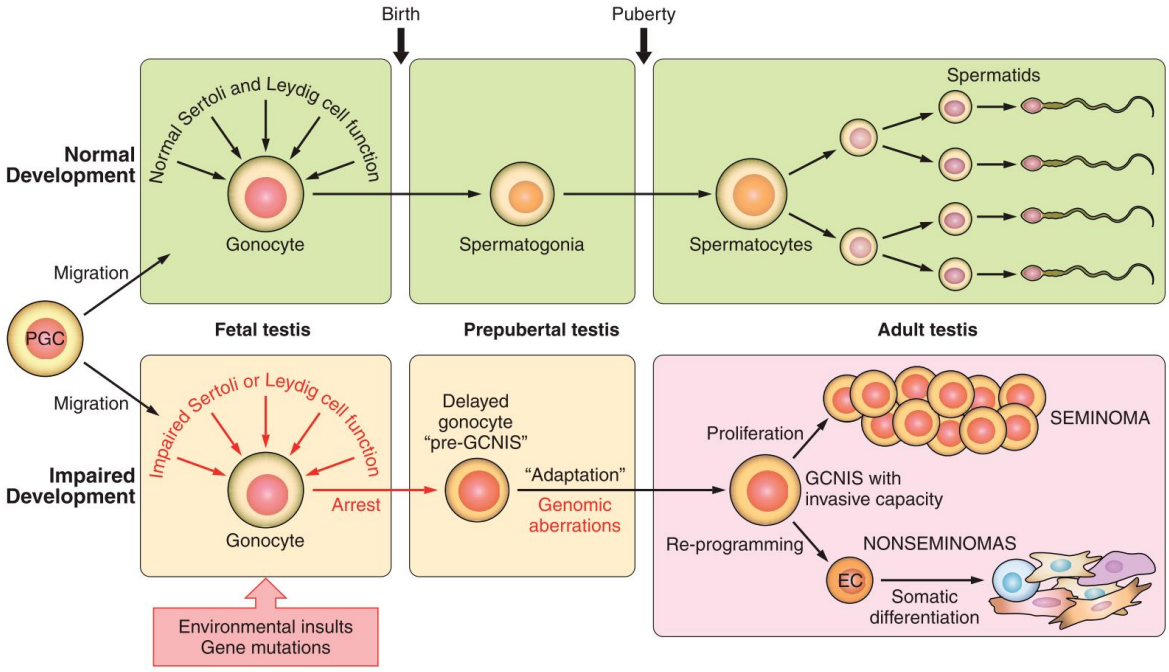
A complex interaction of environmental influences in fetal and early life, specific single nucleotide polymorphisms (SNPs) and epigenetic changes are involved in TC development.[13,14,16-21] The familial clustering of TC, as well as the increased risk of developing a metachronous contralateral TC, supports the hypothesis of a strong genetic component.[22,23] Among all cancers, TC is associated with one of the highest familial risks.[14] Brothers of TC patients have a 4 to 10-fold increased TC risk, while sons of TC patients have a 2 to 6-fold increased risk.[24-26] A family history of other cancers are also associated with an increased TC risk, supporting a component of hereditary cancer syndromes.[27,28]

The contribution of genome-wide association studies and the identification of SNPs have been crucial for the current understanding of the complex genetic susceptibility of TC.[16,18,20,29-32] Multiple susceptibility loci, associated with chromosome segregation, DNA repair mechanisms, maturation and differentiation of male germ cells and KIT-MAPK signaling, have been identified.[18,21] A strong association with TC has been found in the KITLG (12q21) locus.[30,32] The KIT/KITLG pathway is critical for germ cell migration to the gonads.[13] Many of the identified genes associated with TC are important for gonadal development and germ cell function,[13] further supporting the hypothesis of TDS.[20] Ethnic differences in frequencies of the KITLG variant,[14,30] as well as the higher TC incidence in Caucasians than Afro-Americans living in the same area, further supports a genetic contribution to TC development.[13] Adult height has been associated with increased TC risk, but genome-wide association studies has not confirmed such associations.[33] Currently, 39% of the familial risk can be explained by independent SNPs,[18] but the understanding within this field is constantly evolving.

The presumed fetal origin of the malignant process is supported by the peak of TC incidence in young adults,[13,14] and the role of environmental risk factors affecting the fetus is supported by the near doubling of TC incidence during the late 20th century in Northern Europe.[34] A birth cohort effect of TC incidence, and the changing TC risk in second-generation immigrants that has been observed in epidemiological studies, also supports in utero or early-life environmental risk factors.[35-37] Exogenous risk factors are, however, not well understood.[14] It is suspected that mother`s exposure to persistent chemicals, like organochlorines, that impair fetal androgen signalling are associated with increased TC risk.[38] The importance of impaired fetal androgen stimulation is further underscored by the increased TC risk in sex development disorders caused by insufficient foetal androgens.[39]

Germ cell neoplasia in situ (GCNIS) is the precursor of TC,[14,40] and if left untreated for 5 years, GCNIS will develop into an invasive cancer in 50% of patients.[41] A theoretical model for the pathogenesis starts with genetic mutations and in utero environmental exposures, together causing an insufficient masculinization of the gonocytes.[13,14] This results in a delayed gonocyte, also called a pre-GCNIS, in the prepubertal testis. Further genetic aberrations strongly associated with TC, including polyploidization and amplification of chromosome 12p,[42] are acquired before and after onset of adulthood, resulting in a malignant transformation from GCNIS to an invasive tumour.[13,14] This process is illustrated in Figure 4, and although it is not yet demonstrated experimentally, the support for this theory is convincing.[43] Further understanding of TC genomics might identify potential targets for novel therapies including immunotherapy in cisplatin-resistant TC.[16]

Figure 4. Pathogenesis of testicular cancer.[13] Permission obtained from The American Physiological Society.



Note: The green background illustrates normal germ cell development.

Abbreviations: PGC, primordial germ cell; GCNIS, germ cell neoplasia in situ.

1.3 Diagnosis of germ cell TC

1.3.1 Presentation and histopathology

Germ cell TC often presents with a painless, unilateral, scrotal tumor.[44] Some degree of pain is however experienced by 10-20% of patients.[44] Further confirmed suspicion of the TC diagnosis is done by scrotal ultrasound and measurement of tumor markers. The confirmation of TC diagnosis is done through a radical inguinal orchiectomy, which also serves as the primary treatment. In some cases, symptoms from metastatic sites initiate the diagnostic process, for instance abdominal pain due to large retroperitoneal metastases.[45] It is import to offer cryopreservation of sperm and to discuss testicular prothesis with the patient before orchiectomy.

The surgical specimen is sent for histopathological examination. Germ cell TC derived from GCNIS accounts for 95% of all testicular malignancies.[40,46] The remaining 5% (lymphoma, spermatocytic tumors, sex-cord stromal tumors, sarcoma, prepubertal non-GCNIS related tumors) are out of scope of this thesis and thus will not be further described.[14,40]

Germ cell TC is divided into two distinct histopathological subgroups; pure seminomas and nonseminomas as defined by the 2016 World Health Organization (WHO) classification.[40] About 55-60% of patients are diagnosed with pure homogenous seminoma histology, which resembles the gonocyte arrested at a pre-differentiated stage.[14,45] Nonseminomas, on the other hand, are heterogenous tumors that may contain a variety of cell types including embryonal carcinoma (resembling undifferentiated stem cells), choriocarcinoma and yolk-sac (both with extraembryonic differentiation), and teratoma.[14,45] Teratomas can in rare cases display a somatic differentiation, histologically resembling sarcoma or adenocarcinoma.[46] Nonseminomas may also contain components of seminoma.

Additional current pathological evaluation includes tumor staging according to the TNM classification 8th edition, and information regarding tumor vascular invasion, stromal rete testis invasion, invasion of tunica albuginea, tunica vaginalis, epididymis or the spermatic cord invasion, and whether GCNIS is present or absent.[15]

There are several clinical differences between the two subgroups. Seminoma patients are generally 10 years older at diagnosis than patients with nonseminoma, with a peak incidence at 35 years.[14] Overall, 85% of seminomas are diagnosed with clinical stage I vs 60% of

nonseminomas.[46,47] With no adjuvant treatment, recurrence will develop in 10-20% of stage I seminoma, and the risk might be influenced by tumor size and whether invasion in rete testis is present.[9] Seminomas metastasize in a predictable, stepwise and relatively indolent manner via the lymphatic system in the retroperitoneum, and visceral metastases are very uncommon.[48,49] Recurrences are generally diagnosed within the first 3 years of follow-up.[50] In stage I nonseminoma, the presence of lymphovascular invasion in the tumor predicts the risk of occult metastases and the risk of recurrence.[51,52] About one-third of tumors are diagnosed with lymphovascular invasion, and if present, the risk of recurrence is 50% in stage 1 nonseminoma if no adjuvant treatment is administered.[47,53,54] Recurrences most commonly occur in the retroperitoneum within 2 years after orchiectomy,[50,53,55]as nonseminomas most commonly metastasize through the lymphatics to the retroperitoneum, and may also continue to supradiaphragmatic lymph nodes.[56] Hematogenous spread of nonseminomas to lung, bone or brain may occur, but is relatively infrequent.[56]

In 2-5% of cases, germ cell cancer presents with an extragonadal localization without a testicular tumor.[57] Extragonadal germ cell cancers were excluded from this study.

1.3.2 Tumor markers

Serum tumor markers include Alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (beta-hCG). At diagnosis, 50-70% of patients with nonseminoma have elevated serum AFP.[58] AFP is secreted by some yolk sac and embryonal carcinomas, while not by pure seminomas or choriocarcinoma. Thus, an elevated AFP is inconsistent with a seminoma diagnosis. Beta-hCG is produced by all choriocarcinomas, 40-60% of embryonal carcinomas and 10-20% of seminomas.[58] The level of beta-hCG produced by seminomas is generally lower than the levels produced by nonseminomas. In nonseminoma, normal tumor markers are more common in stage 1 vs. metastatic disease; In stage I, 45% have normal levels of AFP or beta-hCG pre-orchiectomy, and both markers are negative in 33%. In metastatic disease, only about 15% have one or both tumor markers normal.[58] The peak levels of tumor markers and the half-life kinetics are important for final staging and treatment evaluation.[46]

Additionally, elevation of lactate dehydrogenase is present in 40-60% of TC patients. It is not specific for germ cell TC, but is used for prognostic classification.[15]

For the last decade, new microRNA-based tumor markers have yielded promising results in TC.[59] The most promising is microRNA (miR)-371a-3p, expressed by both TC and GCNIS tissues, and superior to the classic TC tumor markers with a sensitivity and specificity of >90% in all histological subtypes except teratoma.[60,61] Further studies to validate this marker for clinical use are ongoing.

1.3.3 Clinical staging

The clinical staging of TC is based on histopathological information, serum tumor markers and computed tomography scans of thorax, abdomen and pelvis (preferably done before orchiectomy). According to the modified Royal Marsden Hospital Staging system as first described by Peckham et al.[62] and later modified by the Swedish and Norwegian Testicular Cancer Group (SWENOTECA),[15] patients are designated to clinical stage I-IV (Table 1).

Table 1. Clinical staging of testicular cancer according to the Royal Marsden Hospital Staging System.[62]

Stage	Description
I	No metastases
Mk+	No metastases, but persistent elevation of serum tumor markers
II	Metastases involving abdominal lymph-nodes. A: <2 cm B: 2-5 cm C: >5-10cm D: >10 cm
III	Metastases involving supradiaphragmatic lymph-nodes. Abdominal lymph-nodes according to stage II (A-D)
IV	Metastases involving extra-lymphatic tissue/organ Abdominal lymph-nodes according to stage II (A-D)

From 1997, the prognostic group classification according to The International Germ Cell Cancer Collaborative Group has also been used to decide upon treatment strategy in metastatic disease (Table 2).[10] According to an update of this classification, 5-year overall survival for nonseminoma patients is 96% in good prognosis group, 89% in intermediate and 67% in poor prognosis group.[63] For seminoma, updated 5-year overall survival is 95% for good prognosis group and 88% in intermediate prognosis group.[64] The inclusion in our study cohort started 16 years before the prognostic group classification was introduced, and information regarding tumor markers was not included in our clinical database. Thus, it was not possible for us to allocate the study cohort according to this classification.

Table 2. Prognostic classification according to the Germ Cell Consensus Classification[10]

Prognosis	**Nonseminoma**	**Seminoma**
Good	Primary tumor in testis or retroperitoneum, No non-pulmonary visceral metastases, and good markers (beta-hCG <5000 IU/L, AFP <1000 µg/L and LDH <1.5 x ULN)	Any primary site, no-non-pulmonary visceral metastases, and normal AFP, any beta-hCG and any LDH
Intermediate	Primary tumor in testis or retroperitoneum, No non-pulmonary visceral metastases, and any intermediate markers (beta-hCG ≥5000 - ≤ 50000 IU/L or AFP ≥1000 - ≤10000 µg/L or LDH ≥1.5 - ≤10 x ULN)	Non-pulmonary visceral metastases
Poor	Primary tumor in mediastinum or non-pulmonary visceral metastases or any poor markers (beta-hCG >50000 IU/L or AFP >10000 µg/L or LDH >10 x ULN)	No seminomas have poor prognosis

Abbreviations: beta-hCG, beta-human chorionic gonadotropin; IU, international units; AFP, alpha-fetoprotein, LDH, lactate dehydrogenase; ULN, upper limits of normal.

1.4 Treatment of germ cell TC

1.4.1 General treatment principles during the study period

In Norway, management of TC is centralized to four university Hospitals. The study participants have been treated in line with the recommendations by the SWENOTECA collaboration,[15,65-67] or according to protocols by the European Organization for Research and Treatment of Cancer and Medical Research Council.[68-76] SWENOTECA treatment protocols have been available for nonseminoma patients from 1980 and for seminoma patients from 2000.[15]

The initial treatment for most participants was an orchiectomy. For stage I nonseminoma, a post-orchiectomy retroperitoneal lymph node dissection (RPLND) was routinely performed as a staging procedure until the early 1990s,[77] when 1-3 cycles of adjuvant cisplatin-based chemotherapy (CBCT) or the surveillance strategy (orchiectomy followed by close monitoring with clinical examinations, diagnostic imaging and measurement of serum tumor markers) were introduced as treatment options (Table 3).[47,65,67,70,75] Abdominal radiotherapy (RT), with gradually reduced target dose from 36-40 Gy to 25.2-27 Gy,[78,79] was the standard treatment for stage I seminoma until the early 2000s,[76] when surveillance or 1 cycle of adjuvant carboplatin became the recommended treatment strategies.[47,80-82]

In case of metastatic disease, CBCT has been the standard treatment for both nonseminoma and seminoma during the entire study period, as it still is today (Table 3).[6,66,83,84] In line with expanding insights regarding treatment efficacy and side effects, the number of CBCT cycles were reduced from ≥4 cycles to 3 cycles for patients with good prognosis (the majority) and 4 cycles for patients with intermediate and poor prognosis from the early 2000s.[7,15,74] For small volume metastatic seminoma, abdominal RT continued to be a treatment option during the entire study period, but the target dose was gradually reduced as described above.

Table 3. General initial treatment principles for TC patients in Norway by decade of diagnosis

Decade	Stage I-IIA	Stage IIB-IV
1980 to 1989	Nonseminomas: staging RPLND (unilateral RPLND only if stage I) followed by CBCT if metastases were histologically verified.[77] Seminomas: adjuvant RT towards paraaortal and ipsilateral iliacal lymph nodes by the L-field technique.[78] The target dose was gradually reduced from 36-40 Gy to 25.2-27 Gy.[78,79] One institution offered RT restricted to the para-aortic area only from 1989.[76]	Cisplatin in combination with bleomycin and vinblastine (CVB), and from 1987 etoposide (BEP), has since the late 1970s been the standard treatment of metastatic nonseminoma and seminoma.[6,66,83,84] Generally ≥4 cycles administered. Some treated according to experimental regimens within research protocols.[68,69,71-74] Nonseminomas: Post-chemotherapy RPLND and surgical removal of additional residual tumors if present in all patients with initial metastatic disease.[66] Further CT if malignant cells present upon histological examination. RT was a treatment option if residual masses persisted after CBCT and/or surgery. Nerve-sparing RPLND from 1989.[77] Seminomas: post-chemotherapy RT of residual masses (until 1986) or surgical removal.[84]
1990 to 1999	Nonseminomas: Primary staging RPLND was abandoned for all patients. Stage I: offered surveillance or 1-3 cycles of adjuvant CBCT.[47,65,67,70,75] Stage Mk+ and IIA: BEP x 3-4 followed by RPLND if residual masses.[85] Seminomas: adjuvant abdominal RT continued as above, target dose usually <30 Gy.	The BEP-regimen remained standard first-line therapy in metastatic disease. From 1995, high-dose chemotherapy with autologous stem cell support was available. Some treated according to experimental regimens within research protocols.[68,69,71-74] Nonseminomas: From 1995 post-chemotherapy RPLND was performed if abdominal metastases >2 cm at diagnosis or in case of residual masses.[85] Seminomas: Surgical removal of residual masses often performed.[84,86-88]
2000 to 2009	Nonseminomas: Stage I: surveillance or one adjuvant BEP cycle.[47,89] Stage Mk+ and IIA: continued as above. Seminomas: Stage I: RT was gradually abandoned. Patients increasingly offered surveillance or 1 cycle of adjuvant carboplatin monotherapy.[47,80-82] Stage IIA: RT still a treatment option.[78]	The number of CBCT cycles was reduced to 3 cycles for patients with good prognosis (the majority of patients) and 4 cycles for patients with intermediate and poor prognosis.[7,74] Seminoma patients offered EP instead of BEP.[78,82,90] Nonseminomas: Post-chemotherapy RPLND continued as above. Seminomas: Post-chemotherapy surgery not recommended.[78,82,87 88]

Note: Clinical stage as described by Peckham et al.[62] Abbreviations: TC, testicular cancer; RPLND, retroperitoneal lymph node dissection; CBCT, cisplatin-based chemotherapy; RT, radiotherapy; Gy, Gray.

1.4.2 Platinum compounds and the retention of platinum

The metal platinum is the most fundamental component of the cytotoxic drugs cisplatin and carboplatin.[91] For 40 years, cisplatin and its analogs have had a considerable impact on the treatment of a number of solid cancers, and it made TC a model of a curable malignancy.[6,91] Genomic features that can explain the chemosensitivity of TC have been identified. Through whole-genome sequencing and functional measurement of apoptotic signaling, primary TCs have been found to have high mitochondrial priming, a trait that facilitates chemotherapy-induced apoptosis.[42]

Cisplatin is a highly-potent cancer drug that interacts with and modifies DNA through intrastrand (>90%) crosslinks in addition to interstrand crosslinks, DNA-protein crosslinks and DNA monoadducts, ultimately leading to apoptosis of cancer cells. [92-94] Germ cell TC is highly sensitive for cisplatin, presumably because of an overexpression of some crucial proteins, decreased repair of DNA-crosslinks and a hypersensitive apoptotic response.[93,95] However, multiple dose-limiting acute side effects are related to cisplatin, such as nephrotoxicity, ototoxicity, neurotoxicity, and nausea.[91] To reduce the risk of renal damage, high fluid intake and sufficient diuresis during treatment is important. Modern antiemetic treatment is effectively minimizing cisplatin-induced nausea. The elimination of cisplatin compounds is mainly renal.[96] Although 50% of cisplatin is eliminated within the first 5 days after infusion, the elimination of cisplatin requires several half-lives that increases with follow-up time.[97,98]

The cytotoxic mechanism of carboplatin is similar to that of cisplatin, although carboplatin requires a much higher drug concentration and longer incubation time to induce the same amount of DNA changes.[99] Carboplatin is considered 4-fold less potent than cisplain.[100,101] The acute toxicity following carboplatin is less frequent and milder than that of cisplatin, except for a larger degree of myelosuppression.[92] Carboplatin is generally excreted as an unchanged drug in the urine.[92]

Traces of platinum have been detected in several organs months after administration,[102,103] and in 2000, Gietema et al. discovered that platinum metabolites could be detected in plasma for up to 20 years after treatment with CBCT.[104] Moreover, serum concentrations up to a 1000 times higher than in unexposed controls have been detected in TCS >5 years after treatment,[96] and up to 10% of the long-term circulating platinum remains reactive.[105] Likewise, adducts have been detected in urine up to 16.8 years after treatment.[96] The retention of platinum is highly relevant for

the development of adverse health outcomes after treatment with platinum-based chemotherapy (PBCT).

1.4.3 Other important cytotoxic drugs in the treatment of TC

Cisplatin has been used in combination with other cytotoxic drugs during the study period. From the late 1970s to 1987, cisplatin in combination with bleomycin and vinblastine (CVB) was the standard treatment.[6,66] Because of less acute neurotoxicity when cisplatin and bleomycin was combined with etoposide (BEP), this has been the standard treatment combination from 1987.[83] From the early 2000s, metastatic seminoma patients were offered cisplatin and etoposide (EP) instead of BEP.[90]

Bleomycin, a glycopeptide antibiotic, acts by forming free radicals that induces DNA cleavage.[91] The elimination of bleomycin is renal. Pulmonary toxicity is the most important dose-limiting toxicity.[91]

Vinblastine is a vinca alkaloid that exerts its anticancer effect by acting upon microtubules, leading to cell cycle arrest in metaphase.[91] The elimination is hepatobiliary, and the most important dose-limiting toxicity is neutropenia.[91] Peripheral neurotoxicity may occur, but milder than for some other vinca alkaloids.[91]

Etoposide is a DNA topoisomerase II inhibitor.[91] DNA topoisomerases are enzymes involved in the transient DNA breaks essential for fundamental biological processes. By poisoning this process, etoposide inhibits the re-ligation of DNA ultimately leading to apoptosis.[91,106] The elimination of etoposide is renal, and myelosuppression is the most important dose-limiting toxicity.[91]

1.5 Follow-up procedures of germ cell TC

The close follow-up after treatment for TC aims to detect relapses as early as possible, and to identify and attempt to ameliorate side-effects after cancer and cancer treatment. Clinical examination, diagnostic imaging and blood samples including serum tumor markers at specific

intervals at the centralized treatment centers in Norway have been recommended by SWENOTECA, with modifications, throughout the study period.[15]

During the study period, all patients were generally followed with controls for a total of 10 years.[15] Due to the usual patterns of relapse,[50] a shorter time interval between controls of 2 months were recommended for the 2 first years after orchiectomy for nonseminoma patients and intervals of 2-4 months for the first 3 years for seminoma patients, followed by longer time intervals between controls for the rest of the follow-up. Towards the end of the study period, a shorter follow-up of 5 years was recommended for nonseminoma stage I treated with adjuvant CBCT, while a total follow-up length of 6 years was recommended for seminoma stage I treated with adjuvant RT, also including longer control intervals and fewer abdominal scans. By the end of the study period, magnetic resonance imaging-scans were recommended in the follow-up of TCS, as concerns grew about an increased second cancer risk after multiple computed tomography-scans.[107,108] Results from a clinical trial concluded that magnetic resonance imaging was noninferior to computed tomography scans in the follow-up of stage I seminoma.[109] As a result of the increased knowledge regarding cardiovascular disease (CVD) risk after CBCT,[110,111] screening for CVD risk factors was recommended in the follow-up guidelines from 2007.[112,113]

Today, follow-up for the majority of patients ends at 5 years after orchiectomy.[15] The recommended control interval for nonseminoma patients is every 3 months for most patients, except stage I with lymphovascular infiltration and intermediate or poor prognosis groups for which 2 month intervals are recommended during the first year of follow-up. For seminoma, 6-month control intervals are recommended from the start of follow-up for the majority of patients. For some seminoma patients the follow-up length is extended to 10 years.

1.6 Surivorship issues for TC survivors

1.6.1 General aspects

Survivorship research seeks to identify and investigate the adverse effects of cancer and cancer treatment aiming to reduce and control these effects in order to enhance the health and quality of life of cancer survivors.[114] In the strictest technical term, adverse treatment effects are defined as long-term if they present during treatment and then persist, whereas late-effects develop months or

years after end of treatment. However, these concepts tend to be somewhat intertwined in the literature. Individuals alive more than 5 years after the primary cancer diagnosis are defined as long-term cancer survivors.

Because of the young age at TC diagnosis and the excellent cure rates even for men with advanced disease, the expected lifespan post diagnosis is 40-60 years.[8,115] Consequently, knowledge regarding survivorship issues is highly relevant in this group of cancer survivors. Research has shown that the survival of TC comes at a cost, and that numerous possibly life-threatening late adverse effects may follow TC treatment.[115] In this chapter I will describe important survivorship-issues faced by TCS, with focus on late effects relevant for this thesis. The chapter is based on the knowledge available at the beginning of the research period for this thesis.

1.6.2 Metachronous contralateral testicular cancer

After a diagnosis of a primary unilateral germ cell TC, the risk of a metachronous contralateral (second) TC is increased with estimated 15 to 20-year cumulative incidences of 1.9-3.9%.[23,116-118] Compared with the general population, the risk of a second TC is 12.4 to 35.7-fold increased.[23,116-121] A second TC is usually treated with orchiectomy, and thus a life-long dependency of testosterone substitution follows this diagnosis.[122,123]

The increased risk for a second TC is presumably explained by shared etiological factors for the first and second TC.[13,19] Based on previous studies, there is an association between young age at diagnosis of the first TC and an increased risk of developing a second TC. [23,116-118,124] Results regarding first TC histology and subsequent second TC risk are inconclusive. [23,118,121,125,126] Cisplatin has been hypothesized to reduce or delay the incidence of a second TC, but literature investigating this association is lacking. Existing literature is either based on public registries without details regarding TC treatment,[23,116] involves individuals treated in the pre-cisplatin era,[117-119] or populations screened for GCNIS.[127,128]

Andreassen et al. investigated the second TC risk in 7102 Norwegian TCS treated during 1953-2007,[116] and they concluded with a 50% risk reduction of a second TC in men with metastatic vs. localized disease in those treated after 1980. This implies a risk reduction related to CBCT. Moreover, Fosså et al. conducted a large register-based study involving 29515 TCS from the US,

and they concluded that future studies should investigate the potential dose-response relationship between cisplatin and eradication of GCNIS.[23]

1.6.3 Neurotoxicity, ototoxicity and Raynaud's phenomenon

By degeneration of dorsal root ganglion, cisplatin can cause peripheral neuropathy that is most often sensory with paraesthesia as the main symptom.[8] Cumulative cisplatin dose is related to incidence and severity, and symptoms of peripheral neuropathy is reported by 20-40% after CBCT.[7,129,130] The neuropathy may become persistent.[7,129,130] Peripheral neuropathy may also develop after treatment with carboplatin, but it is infrequent with symptoms in only 3% of patients.[92] Vinblastine is also associated with some degree of peripheral neuropathy, causing paraesthesia in 7-31% of patients.[91]

By damaging of the outer hair cells of the cochlea, cisplatin can induce tinnitus and hearing loss.[131] Persistent hearing impairment has been reported in 20% of TCS after standard cisplatin doses with increasing prevalence after higher cumulative doses.[130,132]

Raynaud`s phenomenon is characterized by white discoloration, coldness and stiffness of digits caused by an abnormal vasoconstriction of digital arteries.[133] This long-term adverse effect is reported by 15-45% after treatment with CBCT, and it is presumed to be a vascular complication brought about mainly by bleomycin,[130,134-136] but cumulative cisplatin also seems important.[130]

1.6.4 Hypogonadism, fertility and sexuality

Gonadal dysfunction in TCS can be observed by lowered testosterone levels (endocrine hypogonadism) and/or oligo -or azoospermia (exocrine hypogonadism).[12] A compensatory increase in serum luteinizing hormone (LH) often accompanies lowered testosterone, while increased follicle-stimulating hormone (FSH) indicate reduced sperm production. Thus, increased levels of serum LH and/or FSH may be the first laboratory sign of hypogonadism.[137] Levels of testosterone below the reference range was observed in 10-17% of long-term TCS with median age of 50 years, depending on treatment, and 50% had levels outside the reference range for either testosterone, LH or FSH.[138] There are several explanations for the frequently observed hypogonadism in TCS,[137-142]

including hypogonadism as a feature of TDS (Figure 3).[13,142] Furthermore, in TCS, the gonadal function is based on only one testicle, and physiological decline of testosterone levels is associated with ageing.[143] Additionally, treatment with CBCT is associated with both endocrine and exocrine hypogonadism.[137-139,144]

In a Norwegian study, long-term TCS treated with CBCT were five times more likely than age-matched controls to have testosterone levels outside the reference range,[138] and increasing Leydig cell deficiency has been observed with higher cumulative doses of CBCT.[137] Endocrine hypogonadism is associated with reduced sexual functioning, loss of energy, muscle weakness, depression, osteoporosis, metabolic syndrome and cardiovascular disease (CVD).[8,115,139,145] Testosterone substitution should be considered for TCS presenting symptoms of hypogonadism together with serum testosterone below the normal range.[9]

Poor semen quality is related to TDS and TC. A more pronounced reduction of sperm quality and concentration, and also changes of sperm DNA, has however been observed in TCS treated with CBCT compared with those treated with surgery.[146,147] One adjuvant cycle of BEP or carboplatin does not seem to influence sperm count.[148] The recovery of spermatogenesis, and thus the ability to father children, has been associated with number of administered CBCT cycles.[147,149-151] Overall, the 15-year actuarial post-treatment paternity rate without the use of cryopreserved sperm was 48% (95% confidence interval (CI) 30%-69%) in TCS treated with high doses of CBCT vs. 92% (95% CI 78%-98%) in the surgery group.[144] Fertility can also be affected by retrograde ejaculation, a possible side effect of RPLND.[8] Offering cryopreservation of sperm is mandatory before starting any TC treatment.

Some TCS experience a reduced sexual functioning compared with controls, and reduced drive, erectile and ejaculatory dysfunction have been observed,[139,152,153] In a Norwegian study, sexual problems was reported by 39% of long-term TCS compared with 36% of controls,[152] however the youngest TCS in this study actually reported a better sexual satisfaction compared with controls.[152] A Danish study did not observe differences in sexual functioning in different treatment modalities, apart from the increased ejaculatory dysfunction after RPLND.[153]

1.6.5 Fatigue, mental health and lifestyle

Chronic fatigue is a subjective feeling of physical, cognitive or emotional tiredness not relieved by rest and sleep, and with a duration >6 months.[154] Chronic fatigue has been reported by 17% of Norwegian long-term TCS compared with 10% in the general population,[155] and the prevalence has been found to increase with increasing follow-up time.[156] Higher levels of circulating interleukin-1 receptor antagonist and c-reactive protein in TCS with fatigue compared with TCS without fatigue has indicated an association with inflammation,[157] but the underlying mechanisms causing fatigue are still not well understood.[158]

Long-term memory problems was reported by 36% of TCS treated with CBCT compared with only 4.3% treated with surgery only, and significantly lower cognitive performance was also observed in the CBCT group.[159] An association between PBCT dose and cognitive decline has also been observed.[160]

Anxiety was more frequent in long-term TCS (19%, 95% CI 17%-21%) compared with age-matched normative controls (14%, 95% CI 13%-14%) in a Norwegian study, and there was a significant association between young age and anxiety.[161] Prevalence of depression was however not higher in TCS compared with controls in the Norwegian study,[161] while an Australian study reported a small, but significant higher prevalence of both anxiety and depression in TCS compared with controls.[162]Increased anxiety has also been observed in TCS after >5 CBCT cycles compared with a lower number of CBCT cycles or surgery.[163] However, a Danish study observed equal long-term quality of life in TCS compared with the general population, and no statistically significant differences were observed between the treatment groups.[134] A Norwegian study observed that a considerable degree of fear of cancer recurrence was common in TCS, and it may persist for many years after the diagnosis.[164] No difference was however observed for the different treatment modalities.[164] Alarmingly, a Surveillance, Epidemiology and End Results (SEER) study including 23381 TCS reported a 20% excess of suicides compared with the general population.[165]

Problem drinking and low intake of fruit and vegetables were reported as more common in TCS than in their age-matched relatives in a US study.[166] Smoking prevalence did however not differ from controls, while engagement in regular physical exercise was higher. Similarly, a Norwegian study, using a sub-population from the current study, also reported comparable smoking prevalence between TCS and age-matched controls, and physical inactivity was less frequent in

TCS.[111] Another US study reported a very low smoking prevalence in TCS of 8%.[167] However, a study involving 7384 cancer survivors, reported a higher prevalence of smoking among younger cancer survivors compared with noncancer controls.[168]

1.6.6 Cardiovascular disease

Atherosclerotic disease was observed in 8% of long-term TCS treated with CBCT compared with a frequency of 3.6% in those treated with surgery in a Norwegian study.[111] This study also reported a significant 3-fold excess risk of myocardial infarction in TCS treated with CBCT compared with age-matched controls.[111] TCS treated with CBCT have been identified as having 1.5 to 2.6 increased long-term relative risks for developing CVD, compared with TCS treated with surgery.[111,169,170] The association between RT and CVD risk has been more conflicting,[111,169] but the combination of CBCT and RT has been found especially harmful in studies with complete TC treatment details.[111,170]

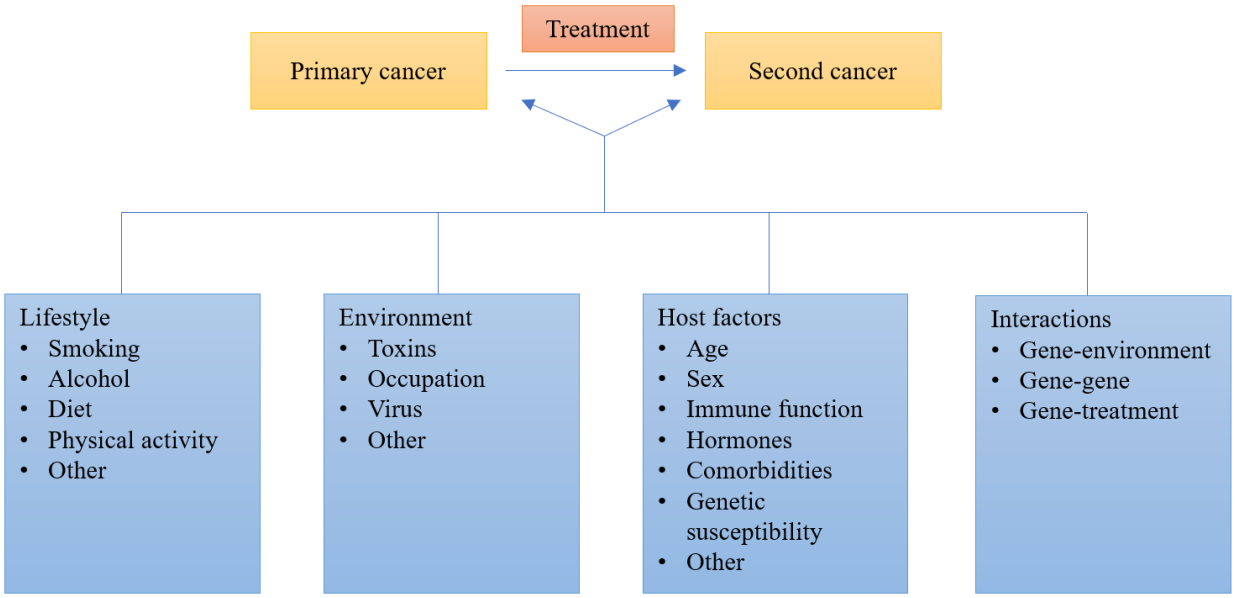
The increased CVD risk after CBCT is presumably caused both by direct endothelial damage and also indirectly by increasing cardiovascular risk factors,[171] e.g. hyperlipidemia[172], hypertension[110,111], obesity,[110] and the metabolic syndrome.[173] RT has been linked with increased risk for diabetes, a possible explanation being radiation injury of the pancreas function.[111] Testosterone deficiency might also contribute to the increase of CVD risk factors.[174]

1.6.7 Non-TC second cancer

It is relatively well documented that TCS have a 1.6 to 1.9-fold increased risk of developing hematological and solid non-germ cell second cancer (SC) compared with age-matched general populations.[119,121,169,175-177] Increased SC risk has been observed after CBCT and RT, but not after surgery only. There is a considerable latency after cancer treatment before SC occur; subsequent hematological malignancies develop within 10 years after cancer treatment,[178,179] while solid neoplasms generally develop beyond 10 years after TC treatment, with risks remaining significantly elevated for at least 35 years.[177] Furthermore, the cumulative risk at any given attained age increases with young age at diagnosis and with increasing follow-up time.[177]

Based on major etiological influences, second primary cancers can be divided in three categories: 1) therapy-related, 2) syndromic, 3) shared exposures.[180] However, multiple factors are usually involved, and the SC risk is often related to the co-existence and interaction of several etiologic influences i.e. previous cancer treatment together with continued tobacco use (Figure 5).[180]

Figure 5. Risk factors for second cancer development. Adapted with permission from Travis, 2002.[181]

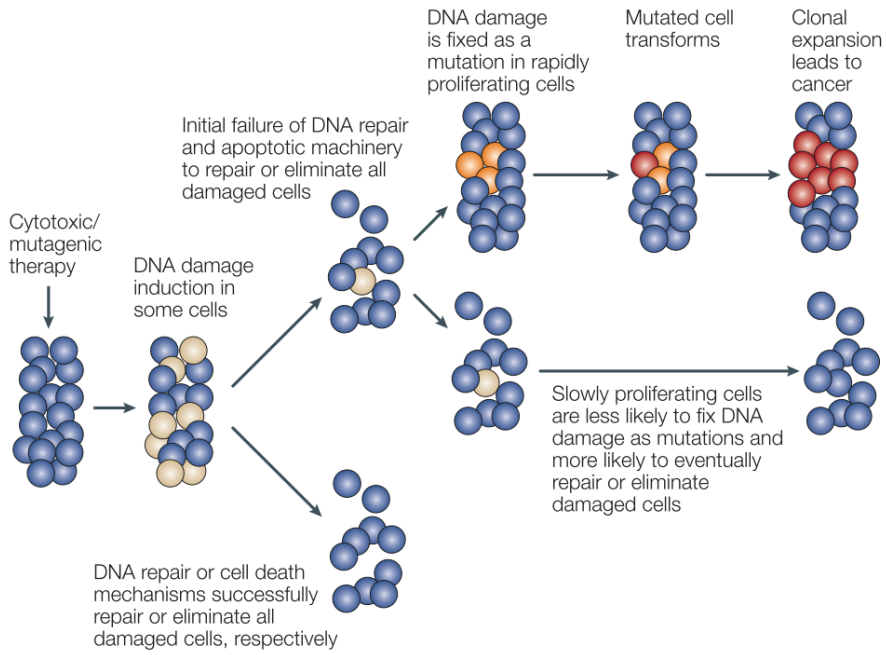


TC has been associated with other familial cancers, as mentioned in chapter 1.2, supporting a hypothesis that TC to some extent is related to hereditary cancer syndromes.[27,28] However, genetic and epigenetic factors related to SCs are complex with individual penetrance of cancer susceptibility genes and their interaction with other etiological factors for SC.[29,182]

Adverse health behaviors are associated with increased cancer mortality in general populations,[183-185] and lifestyle behaviors like smoking and alcohol has been suggested to contribute to 35% of excess SC risk in a report involving two million cancer survivors within the SEER registry.[186]

Therapy-related cancers are defined as SCs that develops after previous chemotherapy and/or RT, and they serve as a potential life-threatening late effect after cancer treatment.[187] Exposure to chemotherapy or RT can induce DNA damage in normal tissue, and if DNA repair mechanisms are affected this may lead to genomic instability which in turn can result in cancer development (Figure 6).[187] For therapy-related SCs, a dose-dependent relationship often exists, and a proliferative state at the time of treatment exposure may influence the SC risk.[187]

Figure 6. The pathogenesis of therapy-related cancers[187] Permission obtained from Springer Nature.



In TCS, there is an established association between treatment with RT and subsequent excess risk of SC.[127,169,177,188] The SCs following RT are often localized in relation to the previous RT field (colon, stomach, pancreas, bladder and the urinary tract).[127,177,188-191]

Experimental data and animal studies have suggested cisplatin as a carcinogen.[192,193] A mechanism found to be involved in cisplatin-related carcinogenesis and resistance, is selection of cells with DNA-mismatch repair deficiency causing genomic instability.[187] Selection of DNA-mismatch repair deficient cells have been observed even after only one exposure to cisplatin in vitro and in vivo.[194]

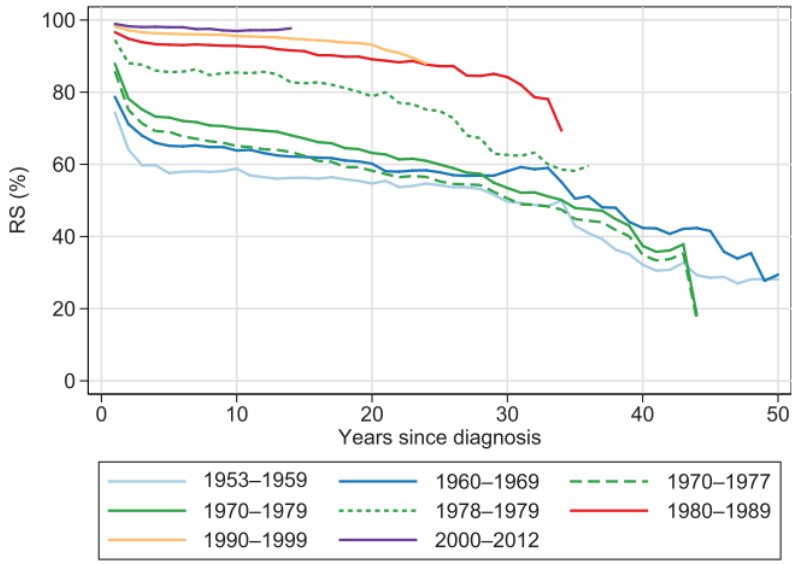
Four decades have now passed since the first TC patients were treated with CBCT, which makes it possible to study very late adverse health outcomes such as SC. Two recent studies have investigated SC risk after modern-era CT in TCS. In 2013, Fung et al. were the first to report of an increased solid SC risk after CBCT in a SEER-based study involving 12691 nonseminoma survivors diagnosed with TC after 1980.[195] Compared with the general population, treatment with CBCT was associated with a 40% overall increased risk of solid SC (standardized incidence ratio (SIR) 1.43, 95% CI 1.18-1.73), and significantly increased risks appeared for cancers of the kidney, thyroid and soft tissue. No increased risk appeared after surgery only (SIR 0.93; 95% CI, 0.76 to 1.14). Details regarding type and dose of initial and subsequent CT were, however, not available for the study population. Kier et al. were the first to include complete information on TC treatment in their study involving 5190 Danish TCS diagnosed 1984-2007.[127] Compared with a control group, they reported significantly 70-80% increased risks of SC after CBCT (hazard ratio (HR) 1.7, 95% CI 1.4-2.0) and RT (HR 1.8, 95% CI 1.5-2.3), but not after surveillance, after median 14.4 years. Three to four BEP cycles were associated with increased risks for SCs of the lung, bladder, oesophagus, soft tissue and for myeloid leukaemia.[127] However, instead of calculating SIRs, Kier et al. estimated cumulative incidences of SC and HRs by using a control group from the general population matched 10:1 on age at diagnosis. Because the majority of available literature are based on outdated TC treatment, studies evaluating SC risk after CBCT are needed.[8,115,196]

1.6.8 Mortality

Mortality due to TC generally occur within the first 5 years after diagnosis, and thus 10- and 15-year relative survival for TC overlaps with rates for 5-year relative survival.[197] Mortality due to TC was not the scope of this thesis. Despite exceptionally high cure rates after TC,[4] long-term relative survival beyond 20 years is inferior in TCS (Figure 7).[198] Compared with general population rates, previous studies have observed an increased non-TC mortality after TC,[198-200] with reported overall 1.3 to 1.6-fold increased mortality risk after PBCT,[127,201,202] and 1.23 to 1.59-fold increased overall mortality risk after RT [127,203,204] while no increased risk has been observed after surgery only.[127,201,202] In line with the increased risk of developing a SC after treatment for TC described previously, deaths due to non-TC SCs have been identified as an important cause of death. After PBCT, the increased SC mortality has been reported as 1.6-fold increased compared with a control

group, and after RT the SC mortality has been reported as 1.6 to 2.4-fold increased, compared with the general population.[188,200,203,204]

Figure 7. Relative survival (RS) of Norwegian TCS by cohort of diagnosis and follow-up time.[198] Permission obtained from American Association for Cancer Research.



Increased risk of non-cancer deaths have also been observed after TC.[127,201,202] Fosså et al. investigated non-cancer causes of deaths in 38907 1-year TCS.[201] They reported an overall standardized mortality ratio (SMR) for total non-cancer mortality of 1.06 (95% CI 1.01-1.10), and significant excess of deaths due to infections, digestive diseases, hypertensive disorders and other respiratory diseases compared with the general population. Further, they reported significantly increased deaths due to circulatory diseases in those <35 years at TC diagnosis (SMR1.23, 95% CI 1.09-1.39) and in TCS initially treated with CT in 1975 or later (SMR 1.44, 95% CI 1.06-1.91).[201] Increased suicide risk after TC has been observed in some previous studies.[165,204] Based on 15006 nonseminoma patients registered in the SEER-database, Fung et al. reported significantly increased overall non-cancer deaths after initial PBCT (SMR 1.60, 95% CI 1.40-1.82), while no increased risk was observed after surgery (SMR 0.96, 95% 0.84-1.11) compared with the general population.[202] They also observed an increased CVD mortality after treatment with PBCT (SMR 5.31, 95% CI

2.65-9.51) restricted to the first year after diagnosis. Kier et al. reported significantly increased risk of mortality due to infection after CBCT.[127]

However, the majority of the available literature investigating mortality after TC lacked complete information on previous TC treatment,[199-202,204] included only patients with localized seminoma treated with RT,[188,203] or included patients treated in the pre-cisplatin era.[201] Despite the complete information on total treatment burden, Kier et al. did not investigate the effect of treatment intensity on mortality risk, nor investigate cause-specific SC mortality.[127]

2 Aims of the thesis

The overall aim of this thesis was to study the associations between TC treatment, with emphasis on cisplatin-based chemotherapy (CBCT), and the subsequent risk of non-TC SC, metachronous contralateral TC and non-TC mortality and causes of death. More specifically the objectives of this thesis were to:

i. Assess the total risk of non-germ cell SC, and the incidence of specific non-TC SCs, among 1-year TCS compared with the general population, with emphasis on the impact of previous TC treatment. Investigate how follow-up time, age at first treatment, histology and treatment intensity, in particular number of CBCT cycles, influenced the SC risk.

ii. Assess the crude and relative risk of developing a metachronous contralateral TC among TCS followed from >2 months after TC diagnosis, with emphasis on the impact of previous TC treatment. Examine how age at diagnosis, follow-up time, histology and treatment intensity, in particular number of CBCT cycles, influenced the second TC risk.

iii. Assess total non-TC mortality and causes of death in TCS followed from TC diagnosis and compared with general population rates, with emphasis on the impact of previous TC treatment. Investigate how follow-up time, age at diagnosis, histology and treatment intensity, in particular number of CBCT cycles, affected the risk of non-TC mortality.

3 Methods

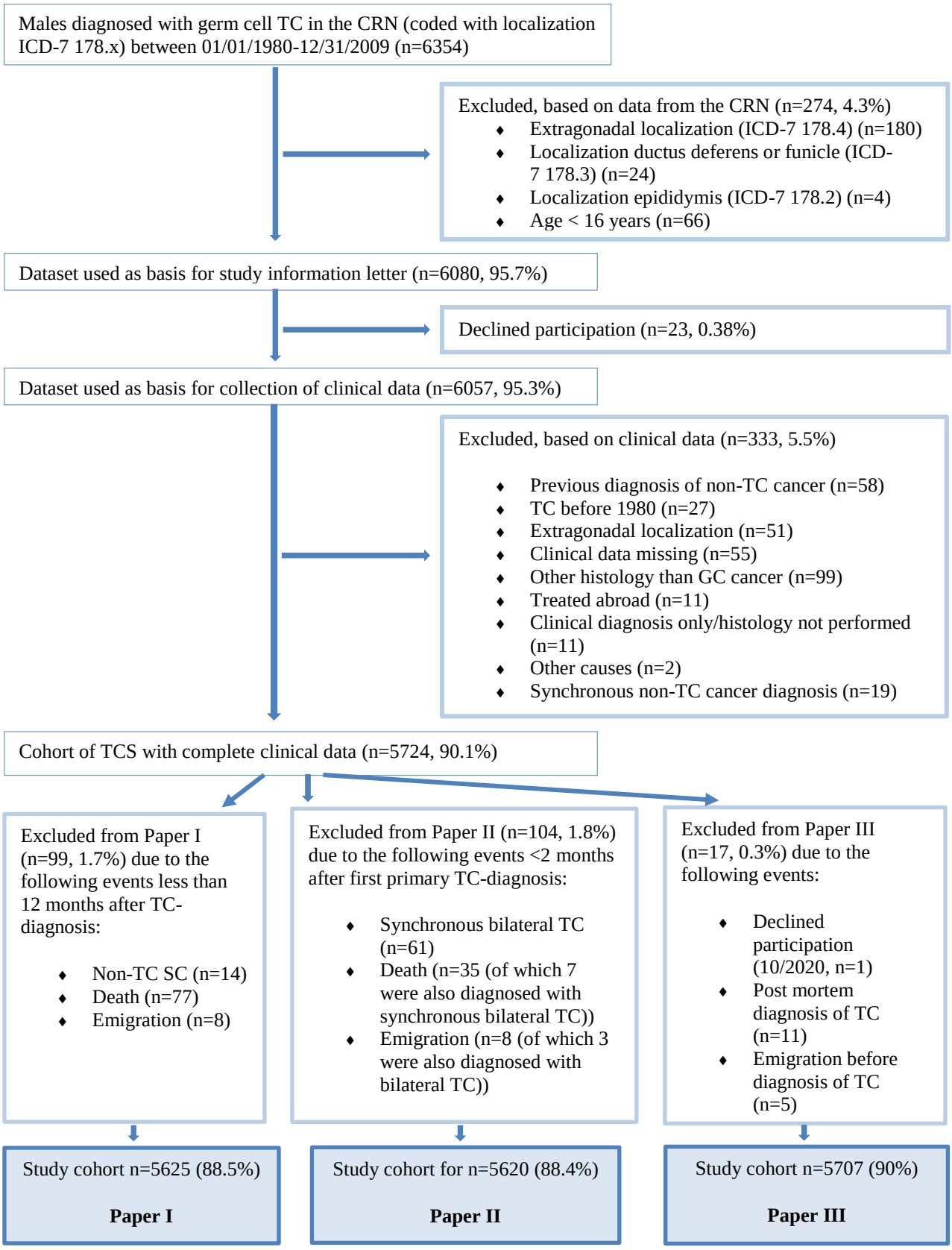
3.1 Study cohort and data assembly

The Cancer Registry of Norway (CRN) identified 6354 males diagnosed with TC between January 1, 1980 and December 31, 2009 based on the International Classification of Diseases (ICD) 7, localization code 178.x (Figure 8). Based on data from the CRN, we excluded individuals coded with an extragonadal localization, or localization in ductus deferens, funicle or epididymis as well as males <16 years at diagnosis (n=274). Of the remaining 6080 individuals, all men alive at the start of the study (n= 5117), received an information letter where they were given the opportunity to withdraw from study participation. Only 23 (0.38%) men declined to participate.

Clinical data were assembled for the remaining 6057 TCS (Table 4). Detailed information regarding initial disease stage, histology, and complete information on all TC treatment, including relapse treatment (Appendix I), was retrieved from medical journals at all four hospitals involved in the post-orchiectomy treatment in Norway. We included all men >16 years diagnosed with germ cell TC from January 1, 1980 and with clinical treatment data available. All men diagnosed with a prior malignancy including TC before January 1, 1980 were excluded. Based on medical journals, a total of 333 men were excluded (Figure 8). The final study cohort comprised 5724 TCS with complete treatment information.

Based on previous studies, clinical data had already been assembled for 2959 participants by the start of this study, and were updated for the present project (Table 3). Clinical data for the remaining 3098 participants were retrieved for this project. Finally, all datasets with clinical data were modified and combined into one master clinical database.

Figure 8. Flow chart presenting the study cohort in the three manuscripts



Abbreviations: TC, testicular cancer; CRN, the Cancer Registry of Norway; ICD-7, International Classification of Diseases version 7, GC, germ cell; TCS, testicular cancer survivors.

Table 4. Assembly of the clinical database

Time period	N (total 6057)	Details
1980-1994	2365	Clinical data complete at the beginning of study, n = 2067 • Participation in the national follow-up survey (all TCS alive minimum 5 years, and no extragonadal germ cell TC, previous malignancy or mental retardation were invited, n= 1814). Responders, n= 1463[110,144,173,205-207] • Included in other projects, n = 604 Assembly of clinical data for this project n= 298 The missing clinical data were retrieved by Hege Haugnes and collaborators
1995-2009	3692	Clinical data were complete at the beginning of this project as part of various study protocols initiated by the SWENOTECA collaboration for n = 892 • Swenoteca III Nonseminoma stage I 1995-2003[89] • Swenoteca IV Nonseminoma metastatic 1995-2012[208] • Swenoteca V Seminoma all stages 2000-2006[82] • Swenoteca VI Nonseminoma stage I 2004-2012[55] • Swenoteca VII Seminoma all stages 2007-2012 Assembly of clinical data for this project, n = 2800 • Oslo University Hospital was not part of the Swenoteca collaboration 1995-2010. Missing clinical data n =1902 (The Norwegian Radium Hospital n = 1519, Ullevål 383) • Seminoma 1995-2000 missing data • Some Swenoteca-data were missing and were retrieved and/or were updated for this project. The missing clinical data were retrieved by Ragnhild Hellesnes and collaborators.

Abbreviations: N, number; SWENOTECA, the Swedish and Norwegian Testicular Cancer Group

3.2 Exposure assessments

Treatment modality and intensity as previously described (chapter 1.4) were the main exposure assessments in all three papers. Further, age at diagnosis/treatment, attained age, follow-up time, and histology served as secondary exposure variables.

3.2.1 Treatment modality

Based on total treatment burden for the first and possibly second TC (paper I and III) or total treatment burden for the first TC only (paper II), the study participants were categorized into four treatment groups, applied in all three papers:

1) Surgery only (including surveillance and, if applicable, additional RPLND)
2) Platinum-based chemotherapy (PBCT). In papers I and II, this group was labeled as the chemotherapy (CT) group. However, for paper III, this group was relabeled the PBCT-group, because only two men of the total study cohort received non-PBCT. In this thesis, the designation PBCT is used when referring to the chemotherapy group as a whole in all three papers.
3) Radiotherapy (RT)
4) Both PBCT and RT (PBCT + RT)

3.2.2 Treatment intensity

In our study, the chemotherapy group as a whole is labeled PBCT, while the label cisplatin-based chemotherapy (CBCT) is used when referring to cisplatin treatment intensity. CBCT is based on total number of CBCT cycles received. In this variable, all chemotherapy cycles containing cisplatin were summarized and accounted for. Thus, participants may have received additional non-CBCT regimens that were not included in this variable. Adjuvant carboplatin monotherapy for stage I and other carboplatin-based chemotherapy were also excluded from this number, but they were included in the statistical models (as separate categories of treatment). The number of CBCT cycles included CBCT administered for the first and second TC in papers I and III, and for the first TC only in paper II.

In paper I, a variable was also constructed based on CBCT regimens containing vinca alkaloids, etoposide or both. For paper II, cumulative doses of CBCT, PBCT and bleomycin administered for the first TC were estimated based on the type of chemotherapy regimen and number of chemotherapy cycles. The cumulative PBCT dose contained cumulative doses of cisplatin and/or carboplatin. For carboplatin, the corresponding cisplatin-equivalent doses were estimated by dividing the carboplatin doses by four was used in the cumulative PBCT dose variable.[101]

RT treatment was categorized and investigated according to first abdominal RT field and corresponding dose categories (1-20 Gray (Gy), 20-29 Gy, 30-39 Gy, ≥40 Gy) for the first or possibly second TC (papers I and III) or for the first TC only (paper II). Ten participants received scrotal RT of a total of 16-20 Gy because of GCNIS or a new tumor that underwent partial orchiectomy. Scrotal RT was not included in the RT group in our analyses.

3.2.3 Age-matched controls

Mortality rates and cancer incidence rates in the general male population of Norway were provided by the CRN and the Norwegian Institute of Public Health. The study cohort was matched with the general Norwegian male population by 5-year age groups and calendar year of follow-up to compare events in the study cohort with general population expected risks. In paper I, a TC-free male general population was used. In paper II, individuals diagnosed with TC was included in the background data, because the diagnosis of second TC was compared with the TC incidence in the general population (see chapter 3.4 for more information). In paper III, TC deaths were not excluded from the background data when examining total mortality due to the nature of data provided by the The Norwegian Cause of Death Registry (NCoDR). However, the proportion of men dying from TC is minimal, so this is unlikely to impact these analyses.

3.3 Outcome assessments

3.3.1 Paper I: Non-TC second cancer

Information regarding non-TC SCs were obtained by linking the clinical database with data from the CRN, updated through December 31, 2016. The CRN provided information on date of diagnosis of subsequent cancers, localization codes (ICD-7), topography codes (ICD-O, third edition), morphology codes (ICD-O, third edition), ICD-10 diagnostic codes, information of metastases at diagnosis and the certainty of diagnosis, vital and emigration status and date of death or emigration, as well as treatment hospital for the primary TC diagnosis. To avoid inclusion of synchronous or SC not likely to be associated with treatment, follow-up started 12 months after the TC diagnosis. Participants who developed a SC, died or emigrated within the first year of follow-up were excluded (Figure 8). Thus, 5625 minimum 1-year TCS were included in this study and followed

until the development of non-TC SC, emigration, death or December 31, 2016, whichever occurred first. A diagnosis of a metachronous TC were not included as a SC.

For analyses regarding specific diagnoses of SC according to the ICD-10 classification, participants were followed until the date the SC of interest occurred. Cancer diagnoses with few cases were grouped when clinically relevant.

3.3.2 Paper II: Metachronous contralateral testicular cancer

Information on metachronous contralateral (second) germ cell TC was obtained from medical records and by linking the clinical database with CRN data updated through December 31, 2018 to ensure complete incidence information. Data from the CRN included the same variables as for paper I. Metachronous TC was defined as a second TC occurring >2 months after the primary TC. Participants with a synchronous bilateral TC, or participants that died or emigrated within the first 2 months after the first TC diagnosis, were excluded (Figure 8). Accordingly, 5620 TCS were included in this study and followed from 2 months after the diagnosis of the first TC until a diagnosis of a second TC, death, emigration or December 31, 2018, whichever occurred first.

3.3.3 Paper III: Mortality and causes of death

Information on mortality and causes of death was obtained by linking the clinical database with data from the NCoDR. The NCoDR provided information on death dates, causes of death for causes IA through II from the death certificate (coded according to ICD-8, -9, or -10), underlying cause of death (according to ICD-8, -9, or -10) as decided in the ICD coding rules, and underlying cause of death according to the European Shortlist (Appendix II), based on the WHO coding rules.[209] The Norwegian Institute of Public Health provides death rates and causes of death according to the European Shortlist for the general population of Norway in the NCoDR statistics bank.[210] Consequently, for causes of death in this study, we used this same variable.

Participants with a post-mortem TC diagnosis or those who were registered as emigrated before the TC diagnosis were excluded from this study (Figure 8). Likewise, one participant withdrew his consent to participate as of October, 2020. Thus, the study cohort in paper III

comprised 5707 TCS followed from the TC diagnosis date, until date of non-TC death or until censoring (date of TC-death, emigration or December 31, 2018, whichever occurred first). TC death was not the scope of this study. Accordingly, mortality refers to total non-TC mortality. For investigations regarding cause-specific mortality, non-TC mortality was divided in two main groups; non-TC SC mortality and non-cancer mortality.

3.4 Statistical methods

Continuous variables were summarized using median and interquartile range (IQR), and categorical variables were presented using absolute numbers and percent. Data were analysed using Stata statistical software (versions MP 14.2 and 16.1; STATA, College Station, TX). A p-value <0.5 was considered statistically significant, and all tests were two-sided. In paper II, differences in median time to second TC among those developing a second TC was tested using the K-sample median test.

Treatment was always analysed as a time-varying covariate to avoid immortal time bias. Immortal time bias refers to a follow-up period during which, by design, the outcome of interest cannot occur.[211] Such bias would occur if we e.g. were to use treatment information available after start of follow-up to classify patients into treatment groups from baseline. This was achieved by splitting follow-up time at exact treatment dates for each treatment group. For instance, a participant accrued observation time in the surgery only group until the date of PBCT or RT if this participant later received such treatment.

Crude cumulative incidences (probabilities) were estimated using the Aalen-Johansen estimator.[212] Deaths of any cause (papers I and II) and TC deaths (paper III) were treated as competing risks. Cumulative incidences with 95% CIs were presented for the total study cohort in all papers and stratified by treatment group (papers II and III), histology (paper I and II) and age at diagnosis dichotomized as <30 or ≥30 years (paper II).

SIRs and SMRs were calculated to compare the observed events of interest in the study cohort to rates in a comparable general population. Estimates were presented with 95% CIs. In paper I, SIRs for total and site-specific SC incidence (if >4 cases observed) in the cohort were achieved by dividing the observed number of cancers in the cohort by the expected cancer incidence in a TC-free, male Norwegian population, matched by 5-year age groups and calendar year of

follow-up. SIRs were estimated for the total study cohort and by treatment groups, age at first treatment, attained age at first SC diagnosis and follow-up time. A subgroup analysis for those initially treated with surveillance was also performed.

In paper II, SIRs were obtained by dividing the number of metachronous contralateral TCs in the study cohort by the expected number of metachronous contralateral TC, given the TC incidence in a male Norwegian population, matched by 5-year age groups and calendar year of follow-up. SIRs were presented for the total study cohort and stratified by treatment group, age at diagnosis, age at diagnosis dichotomized (<30 or ≥30 years), histology and follow-up time.

In paper III, SMRs were obtained by dividing the observed number of deaths in the cohort by the expected number of deaths in the general Norwegian male population, matched by 5-year age groups and calendar year of follow-up. SMRs were presented for the total study cohort and stratified by treatment group for total non-TC mortality, non-TC SCs and groups of non-cancer causes of deaths if >4 observed, follow-up time, age at diagnosis and attained age. In paper III, absolute excess risks (AERs) were calculated as the absolute difference in mortality rates using the following formula: [(observed number of deaths – expected number of deaths)/person-years of observation]*10000.

Cox regression models adjusted for age at first TC diagnosis, with time since diagnosis as time scale and the surgery group as reference, were used to evaluate the impact of treatment group, treatment intensity and histology in all three papers. In paper II, histology was further evaluated in a multivariable Cox regression model including treatment in addition to age at diagnosis, and in paper III, all Cox regression analyses were performed in multivariable models including histology in addition to age at diagnosis. Estimates were presented as HRs with 95% CIs. In paper I and II, when effects of number of CBCT cycles were evaluated, all participants that received RT were censored at the RT start date. Similarly, when investigating effects of first RT field and abdominal RT dose, all participants that received PBCT were censored at the date of first PBCT treatment. In Paper III, all treatment options were included in the model for analyses regarding treatment intensity.

For paper I and III, time-dependent Cox regression models were applied because the proportional hazard assumption was violated for most analyses and, importantly, because a 10-year cut off was deemed clinically relevant based on previous research.[177,198,201] Consequently, treatment (papers I and III) and histology (paper III only) were analyzed as time-varying coefficients. This

was obtained by including an interaction with follow-up time dichotomized as up-to and after 10 years. Unless otherwise specified, results were presented for those with >10 years follow-up time in Papers I and III, or starting 1 year from TC diagnosis (Paper I).

In paper II, the proportional hazard assumption, judged by a nonsignificant Schoenfeld residuals test, was met for all analyses, except for some analyses regarding cumulative CT doses. For the latter, new models were fitted with an interaction effect between follow-up time and the affected cumulative doses, and model fits were compared using Bayesian Information Criterion. The best fit was provided by the simple model without interaction effects for all analyses, and hence, the age-adjusted Cox proportional hazard regression models were applied. Also, in paper II, the Cox regression model was used to investigate the effect of age at first TC diagnosis (dichotomized on <30 and ≥30 years). For this analysis, the Schoenfeld residuals test was significant (p=0.03), but the visual inspection of the log-log survival plot was judged to meet the proportional hazard assumption for this analysis.

Kaplan-Meier failure curves adjusted for age at TC diagnosis centered on mean were estimated to illustrate the effects of number of CBCT cycles (all papers), treatment group for the whole study cohort (papers I and III) and treatment group according to histology (paper I). Risk tables presenting crude number of individuals by follow-up time accompanied the Kaplan-Meier plots in papers II and III. In paper III, unadjusted Kaplan-Meier curves including population expected (PE) risks were also included. PE risk was calculated from population lifetables containing mortality rates stratified by 1-year age groups and calendar year.

3.5 Ethics and approvals

The study was approved by the Data Protection Authorities at the University Hospital of North Norway (2015/2008) and the Regional Committee for Medical and Health Research Ethics (2014/1745), with the condition that all men still alive at the beginning of the study were given the possibility to withdraw (passive consent). Passive consent was obtained through a study information letter distributed to all eligible men still alive, in which they were given the possibility to withdraw from participation (Appendix III).

4 Main results

4.1 Paper I

Continuing increased risk of second cancer in long-term testicular cancer survivors after treatment in the cisplatin era (Hellesnes et al., Int J Cancer 2020)

With information on total treatment burden available, the aim of this population-based study was to examine the SC risk with emphasis on the impact of previous TC treatment.

Median follow-up time for the total cohort of 5625 1-year TCS was 16.6 years (IQR 10.9-23.8), median age at diagnosis was 32.9 years (27.1-40.7) and seminoma histology was present in 52% of the participants. Over the decades from 1980-2009, the use of surgery and PBCT increased, and RT decreased.

Overall 572 (10.2%) participants developed 651 non-TC SCs. Compared with the general population, the TCS had an overall 58% excess risk of developing any SC (SIR 1.58, 95% CI 1.45-1.171), and an overall 44% excess risk of developing a solid SC (SIR 1.44, 95% CI 1.32-1.57). The overall excess risk of all hematological malignancies was also increased (SIR 1.31, 95% CI 1.00-1.71). The overall SC risk was significantly 1.28 to 2.00-fold increased for all treatment groups including surgery only. For TCS initially intended for surveillance, the total SIR was 1.34, (95% CI 1.07-1.68). After surgery, excess risks emerged for melanoma (SIR 1.94, 95% CI 1.10-3.43) and cancer of the thyroid (SIR 4.95, 95% CI 1.86-13.18). After PBCT, significantly 1.86-3.73- fold excess risks emerged for cancers of the small intestine, lung, melanoma, kidney and bladder. After RT, significantly 1.47 to 4.43-fold excess risks emerged for cancers of the stomach, small intestine, liver, pancreas, lung, kidney and bladder.

The overall SC risk increased with increasing follow-up time from SIR 1.28 (95% CI 1.09-1.51) for those with <10 years follow-up time to SIR 2.12 (95% CI 1.55-2.90) for those with follow-up time 30-37 years. After surgery only, significantly increased SC risk was only present with follow-up <10 years, while the opposite was observed after PBCT and RT with significantly increased SC risk observed after >10 years of follow-up. The SC risk was highest in those <20 years at first TC treatment (SIR 2.29 95% CI 1.09-4.80), particularly those treated with PBCT (SIR

3.17, 95% CI 1.43-7.06). It declined with increasing age at first TC treatment to SIR 1.39 (95% CI 1.19-1.63) in those >50 years.

The 25-year crude cumulative SC incidence was 20% (95% CI 18%-22%) after seminoma and 10% (95% CI 8.7%-12%) after nonseminoma. Compared with the general population, the SC risk was similarly increased after both seminoma (SIR 1.59, 95% CI 1.44) and nonseminoma (SIR 1.55, 95% CI 1.35-1.77). Compared with nonseminoma, seminoma histology was associated with increased SC risk in an age-adjusted model (HR 1.20, 95% CI 1.01-1.44).

Compared with surgery, the total SC risk was significantly 1.6 to 1.7-fold increased after PBCT, RT and PBCT+RT after >10 years of follow-up. The hazards for solid SCs were even more pronounced, while hazards regarding hematological malignancies were not statistically significant. Significantly increased SC risks were observed after 2 (HR 1.91, 95% CI 1.01-3.59), 4 (HR 1.60, 95% CI 1.12-2.30) and >4 (HR 2.09, 95% CI 1.23-3.53) CBCT cycles, compared with surgery after >10 years of follow-up. No difference in SC risks emerged when cisplatin was combined with vinca alkaloids vs. etoposide. Compared with surgery, both RT treatment with the L-field technique and paraaortic RT were associated with 1.7-fold increased SC risk, and increased SC risk was observed after RT doses of ≥20 Gy to first abdominal field, after >10 years of follow-up.

In conclusion, all treatment modalities were associated with an increased non-TC SC risk compared with the general population, but the risks were particularly increased after PBCT and/or RT. Treatment with ≥2 CBCT cycles was associated with increased SC risk.

4.2 Paper II

Metachronous contralateral testicular cancer in the cisplatin era: A population-based cohort study (Hellesnes et al., J Clin Oncol, 2021)

Studies investigating the risk of a second TC in relation to previous TC treatment are lacking, and in this paper, we aimed to assess the metachronous contralateral TC risk, emphasizing the impact of previous TC treatment.

The study cohort comprised 5620 TCS with >2 months follow-up and with a median observation time of 18.0 years (IQR 12.0-25.5). Median age at first TC was 33.0 years (IQR 27.2-

40.9) for the total study cohort, and 38% were <30 years. Overall, 70% were diagnosed with stage I at first TC, and 25% were treated with surgery while 44% were treated with PBCT at first TC.

In total, 218 (3.9%) men were diagnosed with a second TC after median 6.2 years (IQR 3.3-10.6), among which median age at first TC diagnosis was 28.7 years (24.6-33.5), and 57% were <30 years. Further, first TC histology was equally distributed with 49% seminomas and 51% nonseminomas, 80% were diagnosed with stage I, and as first TC treatment 33% had surgery only and 32% had PBCT. Histology of second TC was seminoma (72 %) in the majority of cases, and 84% of second TCs were diagnosed with stage I.

The overall 20-year crude cumulative incidence of a second TC was 4.0% (95% CI 3.5-4.6), with a lower incidence in those ≥30 years (2.8%; 95% CI 2.3-3.4) at first TC diagnosis than in those <30 years (6.0%; 95% CI 5.0-7.1). The incidence was also lower after treatment with PBCT (3.2%; 95% CI 2.5-4.0) than after surgery only (5.4%; 95% CI 4.2-6.8) or RT (4.5%; 95% CI 3.6-5.6).

Compared with the risk of developing TC in the general population, the risk of developing a second TC was 13-fold higher (SIR 13.1, 95% CI 11.5-15.0). Also, compared with the general population, the risk decreased with increasing age at first TC diagnosis and decreased with increasing follow-up time.

The second TC risk was significantly lower after treatment with PBCT at first TC (HR 0.55, 95% CI 0.40-0.76), with surgery only as the reference group. For each additional CBCT cycle administered, the second TC risk decreased with significantly reduced risk after 3 (HR 0.53, 95% CI 0.29-0.97), 4 (HR 0.41, 95% CI 0.25-0.66), and >4 cycles (HR 0.21, 95% CI 0.07-0.66). Adjuvant carboplatin monotherapy was not associated with a reduction of second TC risk (HR 1.22, 95% CI 0.62-2.39). RT treatment did not influence the second TC risk (HR 1.10, 95% CI 0.79-1.54).

There was no difference in second TC risk related to first TC histology when treatment at first TC was included in the Cox regression model (HR 0.97, 95% CI 0.65-1.45). Age ≥30 years was associated with a significantly decreased risk of second TC (HR 0.47, 95% CI 0.36-0.62).

In conclusion, we observed a strong association between number of CBCT cycles and second TC risk, with significantly reduced second TC risks after >2 CBCT cycles. Age at first TC

diagnosis also influenced the second TC risk, while histology did not affect the second TC risk when adjusting for TC treatment.

4.3 Paper III

Testicular cancer in the cisplatin era: Causes of death and mortality rates in a population-based cohort (Hellesnes et al., revised manuscript under review in J Clin Oncol)

In this paper, we investigated the impact of modern TC treatment on cause-specific non-TC mortality.

Median follow-up time for the total study cohort comprising 5707 TCS was 18.7 years (IQR 12.7-35.0), and 46% had follow-up time beyond 20 years. Histology was equally distributed with 52% seminoma and 48% nonseminoma. In total, there were 665 (12%) non-TC deaths. The median age at TC diagnosis for the whole study cohort was 33.1 years (IQR 27.0-40.8), while those registered with non-TC death were older (median 44.6 years, IQR 34.9-54.7).

The overall 25-year crude cumulative non-TC mortality was 13.7% (95% CI 12.5-14.9), whereas the population expected (PE) risk was 11.3%. The 25-year cumulative non-TC mortality in the cohort and the PE risks were similar after surgery (10.1%, 95% CI 8.0-12.4 vs. PE risk 10.6%), while it was higher after PBCT (9.5%, 95% CI 7.9-11.3 vs. PE risk 8.1%), RT (19.0%, 95% CI 16.8-21.2 vs. PE risk 14.9%), and PBCT+RT (18.4%, 95% CI 13.3-24.2 vs. PE risk 14.1%).

The overall non-TC mortality in the study cohort was significantly increased compared with the general population (SMR 1.23, 95% CI 1.14-1.33, AER 11.14). Significantly 1.23 to 2.04-fold increased excess non-TC mortality emerged after treatment with PBCT, RT and PBCT+RT, while no excess appeared after surgery only. The non-TC mortality risk was highest in those <20 years at TC diagnosis (SMR 2.27, 95% CI 1.32-3.90, AER 14.42), especially in those previously treated with PBCT (SMR 2.49, 95% CI 1.29-4.78, AER 16.55). The non-TC mortality risk increased with increasing follow-up time beyond 10 years.

Overall, 257 (4.5%) participants died as a result of non-TC SC. Compared with the general population, the TCS experienced a 53% excess non-TC SC mortality risk (SMR 1.53, 95% CI 1.35-1.73, AER 7.94). The SC mortality risk was not increased after surgery only, while 1.43-3.24-fold

excess risks appeared after PBCT, RT and PBCT+RT. After PBCT, 1.69 to 6.82-fold excess SC mortality emerged due to cancers of the lip/oral cavity/pharynx, esophagus, lung, bladder and leukemia compared with the general population. After RT, 3.02 to 4.91-fold excess SC mortality was observed for cancers of the lip/oral cavity/pharynx, stomach, liver, pancreas and bladder.

In total, 408 (7.1%) participants were registered with non-cancer deaths. The risk of non-cancer mortality was significantly increased compared with the general population (SMR 1.15, 96% CI 1.04 to 1.27, AER 4.71), with SMRs of 1.17 to 1.55 following PBCT, RT and PBCT+RT. PBCT was associated with significantly increased suicide risk (SMR 1.65, 95% CI 1.01-2.69, AER 1.39), and RT was associated with significantly increased mortality due to diseases of the digestive system (SMR 2.46, 95% CI 1.59-3.82, AER 3.29). The overall CVD mortality was not increased in the study cohort compared with the general population. However, within the first year of follow-up, death due to CVD was significantly elevated after PBCT (SMR 3.90, 95% CI 1.26-12.08, AER 10.42).

Compared with surgery only, the total non-TC mortality risk was significantly 1.42 to 2.79-fold increased following PBCT, RT and PBCT+RT after >10 years of follow-up. The risk for non-TC SC mortality was even more pronounced with estimates 1.69 to 3.95-increased after PBCT, RT, and PBCT+RT. In multivariable models adjusting for histology and treatment in addition to age at diagnosis, the non-TC mortality risk following PBCT remained unchanged, while minimal changes were observed for the risks following RT and PBCT+RT. Additionally, histology did not influence the non-TC mortality (HR 0.93, 95% CI 0.71-1.23) in the multivariable model. Compared with surgery, increased non-TC mortality appeared after ≥3 CBCT cycles, with significant hazards after 4 (HR 1.41, 95% CI 1.01-1.99) and >4 (HR 2.04, 95% CI 1.25-3.35) cycles beyond >10 years of follow-up. RT with the L-field technique or paraaortic RT was associated with 1.48- to 1.60-fold increased non-TC mortality, and abdominal RT doses of ≥30 Gy for non-TC mortality and ≥20 Gy for SC mortality were associated with significantly increased risks after >10 years of follow- up.

In conclusion, TC treatment with PBCT and/or RT is associated with significantly increased premature non-TC mortality, and in particular SC mortality, compared with the general population. The highest non-TC mortality risk was observed in the youngest TC patients. Increased risk of mortality appeared after >2 CBCT cycles in comparison to surgery only.

5 Discussion

5.1 Methodological considerations

All three papers in this thesis are based on the same population-based cohort identified by the CRN, diagnosed with TC 1980-2009. Thus, by referring to "the study" in the following discussion of methodological consideration, this applies for all three papers. If some parts of the discussion are only relevant for one or two papers, this is specified in the text.

5.1.1 Study design

The design of this epidemiological study is a cohort study. With this design, a group of people are identified, often based on exposure status or involvement in a defined population group, and then followed over time to capture the occurrence of health-related events.[213,214] Thus, this observational study design is well suited to study the natural history of suspected risk factors and associate them with future outcomes. Both absolute and relative risks can be measured by this design, and if population-based, incidence rates can be deduced to similar populations elsewhere.[214] Comparisons of incidence of within-cohort subgroups that differs in levels of exposure are common in cohort studies.[213]

Cohort studies can be prospective (synonyms: concurrent) or retrospective (synonyms: historical prospective, nonconcurrent prospective, prospective study in retrospect, historical).[213] In a prospective design, the cohort is assembled at present and then followed or traced for a period of time towards the future. The advantage of a prospective design is that the data collection is fitted to meet the study`s objective, however they are expensive and time-consuming.[214] Additionally, prospective cohorts are very rarely based on a representative population, and a satisfactory follow-up can be difficult to maintain as censoring can occur based on many factors other than the obvious factors death or emigration.[215] The retrospective design, on the other hand, use existing records (i.e. relevant features of a population as they were at some time in the past) without regard to the outcome status, and trace the population forward up to, and possibly including, the present to investigate the occurrence of the outcome of interest.[216] In other words, it is conducted by reconstructing data about persons at a time or times in the past.[213] This design is often used in relation to record linkage (the combination of information from two or more records by the use of a unique identifying system such as personal identification numbers),[213] and it is the chosen design

for a successful register-based study.[217] This design has made important contributions to scientific comprehension of disease causation.[213] Strengths of the retrospective design is the low cost and speed. A main limitation is the obligatory reliance on the quality of preexisting information, i.e. the exposure and/or outcome information may not be suitable to fulfill the study objectives.[214,215] According to Bhopal, the difference between the retrospective and the prospective cohort study is minimal, it is merely the use of historical vs present records on exposure status.[214] Nevertheless, the terms have been widely discussed and created confusion, and as a solution it has been suggested that describing what has been done instead of labeling the study.[218]

The present study is a retrospective cohort study. However, of the many available synonyms, the term historical prospective cohort study best captures the design of this study. As already mentioned, the cohort is population-based, identified by the CRN. The cohort was assembled based on a common feature; the diagnosis of germ-cell TC between 1980-2009 (in the past), and the exposure status (TC treatment) was based on historical medical records. The study population was then followed until the occurrence of an event obtained by linkage with the CRN (papers I and II) and the NCoDR (papers III) or until emigration or the end of study. The design of the study made it possible to report incidence rates that can be extrapolated to similar populations elsewhere and also make within-group comparisons of the different exposures (treatment groups).

The causal inference in epidemiologic research, for which Sir Bradford Hill`s considerations remain a cornerstone, focus on whether confounding or bias are possible alternative explanations for an observed statistical relationship, and if they are not, whether a cause-relationship can be assumed.[215] The golden standard for causal inference in epidemiology research is experimental evidence (randomized controlled trials), however for many clinically significant research questions, conducting a trial is unethical or not possible. Temporality is another consideration for causal inference; the exposure always precedes the outcome. This is a strength of prospective cohort studies, and although historical, the study design of the papers in this thesis has a distinct temporality. Other considerations of causal inference include strength of the association, dose-response relationship between exposure dose and risk of outcome and the existence of a biological plausible explanation. It is important to bear in mind that causal inference cannot be drawn based on results from a single observational study. Consistency of an association across epidemiological studies is a consideration of great importance, and it is the rationale behind the meta-analytic techniques aiding policy decision making.[215]

5.1.2 Validity

Validity refers to whether the inferences drawn from a study are valid, and as such validity relates to the quality, or the lack of errors, in the entire process of the study.[213]

External validity refers to the degree of generalizability of results from a study to other populations or groups that did not participate in the study.[213] Generalizability can be improved by conducting strict inclusion or exclusion criteria and other strategies that limits confounding.[219] In our study, the entire Norwegian population with TC was included. Except for the 23 men (0.38%) that declined to participate, the population in the study was complete, and thus representable for the Norwegian population. We did not have information on race for our subjects, however, the proportion of non-white participants is probably very small.[220] Thus, the results in this study is presumably not generalizable to non-white populations, but it is considered generalizable to white populations in other countries with similar availability of health care and TC treatment. Though the TC treatment have been modified somewhat during the last decades (discussed in chapter 1.4.1), the exposure variables (TC treatment) in this study are considered highly generalizable to treatment as it is today. Albeit adjuvant RT is no longer recommended in Norway, this treatment is still in use in some other countries,[221] and as we expect the SCs and mortality associated with RT to persist for yet another decade,[177,198] this exposure is also still relevant for TCS in Norway. Likewise, the outcomes (SC, second TC and mortality) are based on national registries with high completeness.[222,223] Altogether, the external validity of our study can be considered as high.

Internal validity concerns to which extent an observed association can be explained by the exposure rather than other alternative factors.[219] Internal validity can be enhanced by minimizing the degree of systematic error (bias and confounding), and will be further discussed in chapters 5.1.3 and 5.1.4.

5.1.3 Bias

Bias can be characterized as a product of systematic error in the design or conduct of a study.[215] The presence of bias will introduce a tendency of deviation from the truth, and thus threaten the validity

of the study. Although there is an abundance of different biases, the majority of biases related to study design and procedures are classified in selection bias or information bias.

Selection bias occurs if there is a systematic error in the recruitment or retention of exposed vs. unexposed study participants.[215] Porta et al., claim that the requirement of informed consent in historical prospective cohort studies threaten these studies, as a large number of participants in reality makes it impossible to obtain an informed consent.[213] The requirement made by the Regional Committee for Medical and Health Research Ethics that all participants still alive were to be informed about the study and given the possibility to withdraw from participating (passive consent), introduced a possible selection bias in our study. If those that withdrew their consent varied according to exposure (TC treatment) or probability of outcome (e.g. if the majority of those that withdrew from participation had experienced a SC) this could introduce a systematic deviation of the results in our study. As only 0.38% men declined participation, the magnitude of this error, if it exists, is considered too small to hamper the results.

In cohort studies, selection bias usually relates to differential losses to follow-up, i.e. the study participants who are lost to follow-up differs from those that remain under observation. If those lost to follow-up have a different probability for the outcome, i.e. that there is not independence between censoring and survival, this can cause bias of the incidence estimates, in particular the estimates of absolute cumulative incidence. As such, independence between censoring and survival is one of two fundamental assumptions in survival analyses.[215] Relative incidence estimates of within-cohort subgroups may however still be estimated if losses to follow-up are fairly similar between exposed and unexposed (a so-called compensating bias). Differential losses to follow-up mainly constitute a problem in prospective cohort studies with long-follow up time. In the present historical prospective cohort study censoring only occurred at death, emigration or study end. The distribution of TC treatment in those that emigrated (n=72 in paper III) was similar to the total study cohort, and thus differential losses to follow-up is negligible in our study. The second assumption in survival analyses is a lack of secular trends during the study`s accrual period.[215] If the characteristics of the participants changed during the accrual period or there were significant changes in exposures (treatment), then bias of cumulative incidence estimates may be introduced. Despite the modifications in TC treatment during the study (as described in chapter 1.4), the TC treatment in this study is still highly relevant today and thus enabling the estimates of cumulative incidences.

Information bias in epidemiological research occurs when the definitions of study variables are inexact or when the data collection procedure is inaccurate.[215] This results in misclassification, defined as a systematic error in the classification of exposure and/or outcome status. Misclassification can be non-differential (random) or differential (non-random). Non-differential misclassification is misclassification of exposure that is independent of the outcome or vice versa, while differential misclassification is misclassification of exposure or outcome that are dependent on status of the other.[215] In our study, misclassification of exposure variables could potentially occur in the process of establishing the clinical database; important information in medical records may have been overlooked, errors might occur whilst punching data into the clinical database, or the information reported in the medical journals was incorrect. These potential errors would cause non-differential misclassification as they are independent of the status of the outcome. These errors may leap in all directions, i.e. information of treatment given might be exaggerated (for example reported as four courses of chemotherapy while in reality none were given to this patient), or it might be understated (for example reported as no additional treatment given while in fact the patient received four courses of chemotherapy). The presence of non-differential misclassification will generally lead to an underestimation of the association between exposure and the disease, and it is an important reason why epidemiological studies underestimate effects. However, unpredictable outcomes may follow misclassification of confounding variables.[214] To minimize the chance of non-differential misclassification during data assembly, clinical data were plotted in a careful and thorough manner. As some degree of measurement error is inevitable,[214] this error could to some degree be reduced if two independent researchers collected the same data. This was however not feasible in our study due to a large amount of data to be collected. From personal experience, the chance for the exposure information in our study (i.e. the TC treatment information) to be flawed in medical records is minuscule.

The outcome information in our papers were obtained from two National Registries. The CRN is a cancer registry with very high completeness; through the Norwegian unique personal identification number, all hospital clinicians, pathology laboratories and general practitioners are instructed by law to report all new cases of cancer to the registry.[222] Additionally, the records in the CRN is supplemented with data from the NCoDR for all deaths registered with a cancer diagnosis to ensure completeness and validity, and with the national population registry for vital status. For information on cancer treatment, however, the quality of the CRN data are considered unreliable.[224] The NCoDR also has a near-complete coverage.[223] However, the quality of NCoDR has been

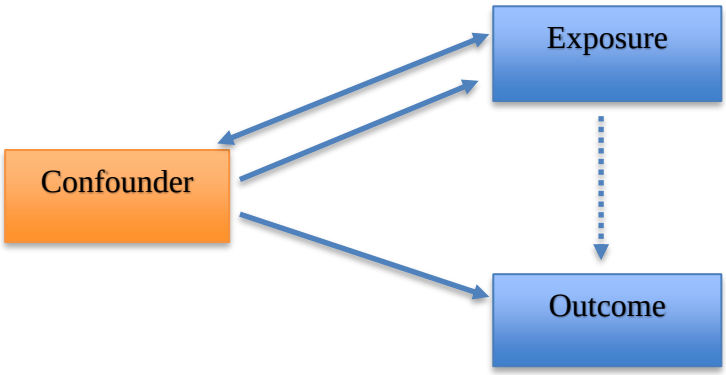
discussed, in part due to the relatively high frequency of unspecified codes for the underlying cause of death.[223] In paper III, 31(3.6% of a total mortality of 846 deaths (including TC death)) were registered with ill-defined or missing causes of death. Additionally, older patients usually suffer from comorbidity and several causes of death may thus be plausible, and autopsies are rarely done. Since 50 % of all deaths in the Scandinavian countries happen after age of 80 years, this is an important reason for why the quality of the NCoDR is questionable.[217] For younger patients, the cause of death is often clear-cut, and autopsy rates are quite high. Errors on cause of death might lead to non-differential misclassification. However, it is possible that a previous medical history of cancer will lead to erroneous use of a cancer diagnosis on the death-certificate when in fact the cause of death is unknown, and this will lead to differential misclassification.

Surveillance bias occurs when exposed individuals are subject to a closer surveillance, and as a result, the detection of the study outcome is more likely in the exposed.[215] In cohort studies, surveillance bias can be considered as a kind of information bias, but it may also be considered a selection bias depending on study design (i.e. case-control study). In paper I, the SC risk after surgery only was highest within the first 10+1years of follow-up, suggesting influence of surveillance bias. Further, no increase of mortality was observed after surgery in paper III, possibly due to early detection However, in the same manner we would then expect the SC risk after PBCT and RT to be increased within the first 10 years of follow up. As this was not the case, we regard that the increased SC risk after surgery was not the product of surveillance bias. In paper II, surveillance bias might have contributed to the majority of second TCs being diagnosed as stage I.

5.1.4 Confounding

Confounding occurs when the association between an exposure and an outcome is influenced by a third variable (a confounding variable or confounder).[215] Thus, an alternative explanation exists for the observed association. A confounding variable must be causally associated with the outcome and also non-causally or causally associated with the exposure, but it is not an intermediate variable in the causal pathway between exposure and outcome (Figure 9). Accordingly, to identify a confounding variable, expert knowledge regarding pathophysiological and clinical mechanisms is required. The association between exposure and outcome might be induced, strengthened, weakened or eliminated by the confounding variable.[215]

Figure 9. The definition of confounding. Adapted from Szklo et al.[215]



Note: A unidirectional arrow indicates a causal relationship and a bidirectional arrow indicates a noncausal relationship.

Once identified, the presence of confounding variables can be managed in two ways: at the planning stage through study design or at the analytical stage through statistical correctional methods. The study design can reduce and to some extent also help avoid confounding. Confounding is much more likely to happen in observational (i.e. cohort studies) than experimental epidemiological studies (i.e. randomized controlled trials).[215] In the latter, the process of randomization, if successful, will produce two groups that are supposed to be similar regarding known confounding factors. In observational study designs, the idea of matching has been introduced as a strategy to try to reduce the effect of confounding factors. Matching is commonly used in case-control studies where cases and controls are picked in a manner so that they are alike regarding confounding factors.[215] Matching is however infrequently used in cohort studies, the main reasons being the large size of most cohort studies and that a multitude of variables regarding exposures and outcomes often are investigated in the same study. Confounding in cohort studies are thus better dealt with at the analytical stage. For this to be feasible, the study must be carefully planned so that possible confounding factors can be accounted for. When a confounding variable is suspected, it can be further assessed and its effect reduced by various statistical techniques like stratification or adjustment in various regression methods, generating corrected or adjusted estimates.[214,215]

Overall, in papers I and III, increasing age is the single most important risk factor for the outcomes (SCs and mortality).[1] In paper II, age is also associated with second TC risk.[23] Additionally, age is associated with the exposure in our study, as those treated with RT (seminomas) are generally older than those treated with PBCT (the majority of which are nonseminomas). Accordingly, age is an important confounding factor in our study. The effect of age on the association was controlled for in all the relevant statistical analyses in the three papers. SIRs and SMRs are methods of indirect age adjustment, and in Cox regression, age was always included in the models. Additionally, in paper II, we stratified on a dichotomized age variable.

Adverse health behaviors like smoking, alcohol abuse, physical inactivity and an unhealthy diet are, as mentioned in chapter 1.6.7, associated with increased cancer incidence and premature mortality.[182-185] As much as 35% of excess SC risk is presumably related to modifiable lifestyle factors like smoking and alcohol.[186] In addition to lifestyle risk factors,[183,225-227] coronary heart disease is also associated with modifiable risk factors like hypertension and hyperlipidemia.[228,229] An important limitation of our study is the lack of information on lifestyle and coronary heart disease risk factors for all participants. Thus, the potential confounding effect of such risk factors on our results cannot be assessed or adjusted for using available statistical methods. The most important lifestyle risk factor related to both SC and mortality is smoking,[182] and consequently I will discuss this risk factor in more detail in the following. As described, a confounding variable must also be related non-causally or causally to the exposure. In the smoking example, this means that the smoking behavior among TCS must differ across the different treatment groups and/or differ compared with the general population. As described in chapter 1.6.5, smoking has not been observed as more common in TCS compared with controls in previous studies,[111,166] while younger cancer survivors had a higher smoking prevalence.[168] However, a recent Danish study observed higher current smoking prevalence among TCS compared with a reference population, with the highest prevalence among TCSs treated with BEP.[230]

It is also possible that underlying genetic aberrations or shared environmental exposures predispose some TCS for other cancers,[27,28,182] or that epigenetic changes brought on by PBCT increase the morbidity in TCS.[231] More research within these fields are needed before their potential roles as confounding variables can be evaluated.

Traits associated with TC as well as presence of GCNIS are potential confounding factors in paper II if they are associated with exposure status (treatment or age) in addition to being related to the second TC risk. The lack of this information constitutes possible limitations in paper II.

5.1.5 Statistical considerations

According to the null hypothesis which states that there is no association between an exposure and the outcome, a **Type I error** is rejecting the null hypothesis when it is true.[215] This error is the most serious, and by tradition, the accepted probability of making a Type I error is <5% in medical research. A **Type II error** is failing to reject the null hypothesis when it is false, i.e. stating that there is no association between the exposure and outcome when in fact there is.[215] The accepted probability to commit a Type II error is 10-20% in most studies. The **statistical power** of a study relates to the study`s ability to detect an association if such exists, i.e. the probability of not making a Type II error.[213,215] The aim in most studies is a power of ≥80%, and this is influenced by factors like sample size, study design, and the frequency of the outcome.

In papers I and III we conducted multiple hypothesis testing when site-specific SC incidence and causes of deaths were analyzed, and this increase the risk of Type I errors. [232,233] There are available methods to reduce the probability of Type I errors in multiple hypothesis testing, and the most frequent method is the Bonferroni correction.[232,233] However, due to the increasing risk of Type II errors, we did not perform such corrections, as advised by Rothman.[234] It is therefore important that caution is taken when interpreting results involving few events.

In paper III, TC deaths were not excluded from the background data provided by the NCoDR when calculating SMRs of total SC mortality. This could potentially lead to an underestimation of the SMRs. However, since TC deaths constitutes a small fraction of the total, this bias is considered negligible.

In regression models, **collinearity** is the presence of a very strong linear relationship between two or more independent variables or covariates.[213] If included in the model, this may result in biased or confounded estimates. The common solution to collinearity is removal of one of the collinear variables from the model, although this might result in confounding if the removed variable is indeed a confounder.[213] When working with paper II we tried to include clinical stage in

the Cox regression model together with treatment group and age. However, all estimates changed dramatically, and because we suspected the reason being collinearity between stage and treatment group, we decided clinical stage could not be included in the model. In paper III, we included histology in multivariable Cox regression models. The estimates regarding RT changed a little bit in the multivariable model, while estimates regarding chemotherapy remained unchanged. The reason for this is probably the collinear relationship between seminoma histology and RT treatment, but because of the still unchanged estimates regarding chemotherapy, we decided to apply the multivariable model because we believe that it was essential to demonstrate that treatment, and not histology, was the most important covariate.

5.2 Discussion of results

In the following discussion of results, when referring to the results in our study, the distinction between PBCT and CBCT described in chapter 3.2.2 is still valid. However, when referring to other studies, the abbreviation CBCT was used when the studies being discussed only studied or mentioned cisplatin, BEP or CBCT.

5.2.1 Metachronous contralateral TC (Paper II)

The overall 20-year crude cumulative incidence of metachronous contralateral TC in the study cohort was 4% and the total SIR was 13.1 which is in line with previous studies.[23,116-121] The increased risk of a second TC is probably explained by shared genetic and prenatal environmental predispositions, as described in chapter 1.2.[13] These shared etiological factors of the first and second TC probably explain why young age at TC has been associated with a significantly increased second TC risk in both previous studies and the present one.[23,117,124,235]

We observed a strong association between PBCT at first TC and a reduced second TC risk, concurring with the hypothesis of a protective role of cisplatin.[23,116,117,119] Further, to the best of our knowledge, we demonstrated for the first time a dose-dependent relationship between number of CBCT cycles and a reduced second TC risk, with significantly reduced risk emerging after ≥3 CBCT cycles. This dose-dependent relationship has also been confirmed by a recent Dutch study,[236] and was also recognized with an editorial in the Journal of Clinical Oncology.[237]

TC is preceded by GCNIS, and if left untreated, 50% of GCNIS will develop into an invasive cancer within 5 years.[41]. In previous studies, the effect of cisplatin on GCNIS eradication has been moderate, but possibly dose-dependent.[128,238-241] Supporting our results, a Norwegian study involving 61 TCS with simultaneous biopsy-proven contralateral GCNIS reported significantly reduced second TC risk after >4 CBCT cycles compared with 1-3 CBCT cycles or no chemotherapy.[128] The need for a sufficient cumulative dose of cisplatin before second TC risk is reduced may in part be explained by the blood-testis barrier`s modulating effect on the intratubular cisplatin concentration.[242,243] The decrease in sperm concentration and changes of sperm DNA observed after CBCT demonstrates that the testicular function is influenced by this treatment.[146,147] Further, the recovery of testicular function is associated with number of CBCT cycles, and a higher probability for long-term reduced sperm count has been observed after ≥3 CBCT cycles[147-151]

The risk-adapted treatment strategy in TC stage I, as recommended by SWENOTECA, enabled the comparison of adjuvant chemotherapy with surveillance.[15] In line with a prospective study,[244] we observed no risk reduction following 1-2 CBCT cycles. Likewise, we did not observe any risk reduction after 1 cycle of adjuvant carboplatin, however this result is contrasted by a randomized trial reporting a reduction of second TC risk after 1 cycle of adjuvant carboplatin compared with RT in 1477 stage I seminoma patients.[80]

Some previous studies have implied that cisplatin, instead of eradicating GCNIS leading to a reduced second TC risk, simply delay the invasive cancer development.[239,240] In line with Schaapveld et al.,[117] our results do not support such observations. In fact, we observed a longer median time interval between first and second TC after surgery (7.0 years) than after PBCT (5.8 years), although not statistically significant. The overall median latency of 6.2 years between first and second TC was identical with the Dutch study.[236]A plateau in second TC incidence has been reported after 15-20 years of observation.[117,118] However, we demonstrate that second TCs may develop more than 20 years after the first TC, with the longest latency between first and second TC of 27 years, in line with previous observations.[245] Despite an equal median follow-up time, no second TCs were observed after 20 years in the Dutch study.[236]

The role of histology on second TC risk has been inconsistent in previous studies; some studies conducted in the pre-cisplatin era have reported a higher risk for metachronous contralateral TC after first TC nonseminoma vs. seminoma.[118,126] In the cisplatin-era, on the other hand, seminoma histology has been associated with a greater risk.[121,125,235] The recent Dutch study also

reported a significantly higher risk for a second TC after first TC seminoma, even when adjusting for age and number of CBCT cycles.[236] The significantly increased risk of the youngest TC patients (i.e. nonseminoma) reported in the Dutch study, is however contradictory with an increased second TC risk associated with seminoma histology.[246] A limitation of the Dutch study was that complete treatment information was available only for those developing a second TC and a sub cohort, corresponding to 19% of the total cohort. In a multivariable model including age and first TC treatment, we observed no association between first TC histology and subsequent risk of second TC, in line with some previous studies.[116,117,124] We suggest that the differences in second TC risk, in some studies associated with histology, merely reflects the fact that nonseminoma patients more frequently are treated with CBCT. Results from studies on contralateral biopsies, reporting that young age and testicular atrophy was associated with presence of GCNIS, whereas histology of a primary TC was not,[247,248] further support this assumption.

Corroborating previous studies,[117,118,124] treatment with infradiaphragmatic RT did not influence the second TC risk. The scattered RT dose to the remaining testicle has been estimated to 0.09-0.32 Gy in stage I seminoma,[249] which is not considered sufficient for eradicating GCNIS.

The majority (72%) of the second TCs in our study had seminoma histology, in line with a review including 51 studies reporting seminoma histology in 60% of second TCs.[250] We hypothesize that the more frequent seminoma histology is an effect of ageing.[1] Robust follow-up procedures, centralized TC treatment, and a risk-adapted biopsy strategy of the contralateral testicle probably accounts for the even higher proportion (84%) diagnosed with stage I in our study, as compared with 73% in the review.[15,250,251] Much anticipation has been associated with the new biomarker miR-371a-3p, however so far it has not proven successful in detection of GCNIS before it becomes invasive.[59]

5.2.2 Incidence and mortality of non-TC second cancer (Paper I and III)

The overall 58% excess SC incidence and the 44% excess solid SC incidence compared with the general population observed in Paper I is in line with a Swedish report investigating SC incidence in TCS treated in the modern era,[252] while it is a little lower than the 80% excess solid SC incidence reported by a Dutch study.[253] A recent SEER-based study reported a SIR of solid SC of only 1.06 in 24900 1-year TCS with a mean follow-up of 15 years, and they suggested that an element of

selection bias due to the hospital-based study design explained the higher SIRs in the Dutch study study.[254] Previous reports involving TCS treated in the pre-cisplatin era reported overall SIR of 1.6 to 1.9.[119,121,169,175-177]

Mortality due to non-TC SCs was the most important cause of death in Paper III, with a total SMR of 1.53. However, the AER of 7.94 was not very high. This is a little lower than the SMR for SC mortality of 1.9 and AER of 19.1 reported by a recent Dutch study investigating mortality in 6042 TCS treated 1976-2006.[255]. In line with our results, a recent report involving 1,5 million cancer survivors concluded that cancer survivors have increased risk of developing or dying from SCs compared with the general population.[256]

In line with previous studies,[177,195,198,253,255] we observed a considerable latency before the risk of SC incidence and mortality increased. The risk of SC and mortality increased with increasing follow-up time, especially beyond 20 years, and this underscores that sufficient follow-up time is required when SC incidence and mortality after TC treatment is studied.

Importantly, in line with previous studies,[177,253,255] an age-gradient emerged for SC incidence and non-TC mortality, with the highest risks observed in those with young age at TC diagnosis. A large SEER-based study identified 5-year adolescents and young adult (AYAs; 15-39 years) cancer survivors, including TCS, as having a higher risk of developing a SC compared with an age-matched general population, and the absolute risk was higher for AYAs than for pediatric or older adult cancer survivors.[257] Thus, it seems that follow-up regarding SC development is particularly important in AYA TCSs.

Contrasting available studies,[127,195,253,254,258] we observed an increased SC incidence after surgery, to our knowledge for the first time. However, no excess SC mortality appeared after surgery, in line with previous studies.[127,255] In fact, the Danish study reported a reduced mortality after surgery (HR 0.9), but as participants were excluded from analysis in case of relapse, we believe that caution must be taken when interpreting these result. Indeed, we observed that participants initially intended for surveillance were associated with a significantly increased SC incidence compared with the general population (SIR 1.34).

Site-specific incidence investigations demonstrated an increased risk for thyroid cancer after surgery. Although based on few observations and a median latency between TC and thyroid cancer

diagnosis of only 5.8 years, which might indicate surveillance bias, this is a novel finding that should be explored in future research. Other studies have reported an increased risk for thyroid cancer after PBCT,[195,253 254] and after RT.[177,254,258] Both thyroid and testicular cancer are associated with endocrine disruptors,[259] suggesting a common etiology.

In line with one previous report,[169] an increased melanoma risk was observed after surgery. Increased melanoma risk has been reported after RT in some previous studies involving TCS,[175,177,260] while the majority of available studies reports no such risk.[127,195,253,254] Increased melanoma risk has been attributed to an increased medical attention during the first years after a TC diagnosis.[260] However, as the median time to melanoma diagnosis in our cohort was 14.6 years, surveillance bias is not a likely explanation for this association.

Recent studies on familial cancer risks have reported a significant association between TC and other cancers, including melanoma and cancer of the thyroid, suggesting the presence of inherited cancer susceptibility syndromes.[27,28,261,262] Likewise, increased SC risk, including TC and thyroid cancer, has been observed in melanoma patients.[263] Additionally, a common susceptibility to BRAF mutation has been reported in melanoma and thyroid cancer, with an observed twofold reciprocal increased risk of developing thyroid cancer after melanoma or vice versa.[264] No TCS developed both melanoma and thyroid cancer in our study. Taken together, we believe the increased SC risk observed after surgery implies that genetic susceptibility and/or common fetal influences predispose for both TC and other malignancies.

The modern era PBCT treatment of TC was associated with a 62% increased SC risk compared with the general population, in line with publications from Denmark, United States, Holland and Sweden.[127,195,252-254]. The Dutch study reported the highest SIR (2.25) after PBCT.[253] PBCT was associated with a 43% increased risk of SC mortality compared with the general population, in line with the Danish study,[127] whereas another Dutch study reported a higher SC SMR (2.54).[255] However, of the available studies, only the Danish nationwide study provided complete information on TC treatment, whereas the two SEER-based US studies provided first-line treatment only.[195,254] The two Dutch multicenter studies provided complete treatment information only for those that developed SC or died and a randomly selected subcohort of approximately 1100 participants, while primary treatment was registered for all participants.[253,255] The Swedish study provided no treatment information.[252]

Bladder cancer emerged as one of the most important SCs after PBCT with a 3-fold increased incidence and a 6-fold increased mortality compared with the general population. The increased bladder cancer incidence after PBCT has been observed in previous studies.[127,169,177,195,253] Increased mortality due to bladder cancer was however not observed in the comparable Dutch study.[255] In line with other reports,[195,252-254] we observed a two-fold increased risk for cancers of the kidney and upper urinary tract after PBCT compared with the general population. We did however not observe an increased kidney cancer mortality, contrasting the Dutch study.[255] Platinum compounds has been detected in urine for up to 17 years after treatment.[96] Thus, a continuing platinum exposure of the genitourinary epithelium to platinum metabolites might explain the increased bladder cancer incidence. As the development of superficial bladder cancer into a more invasive cancer takes many years, a long observation time is needed before mortality is observed.[265]

We have reported that two or more CBCT cycles were associated with an increased SC incidence, and three or more CBCT cycles were associated with increased non-TC SC mortality, consistent with the two Dutch studies.[253,255] A strength of the Dutch studies was the inclusion of smoking status at TC diagnosis in the multivariable models investigating treatment intensity. As demonstrated in Paper III, the follow-up time was shorter for 3 CBCT cycles, which probably explains why statistical significance was not reached for those estimates. In line with a previous study,[266]adjuvant treatment with one course of CBCT or Carboplatin was not associated with an excess SC incidence nor mortality, however the follow-up time is still short necessitating future studies.

Consistent with the increasingly extensive documentation of excess SC incidence and non-TC SC mortality after RT in TCS, [127,169,177,188,204,253-255,267] we reported an overall SIR of 1.64 and SMR of 1.59 after previous treatment with RT. We observed significantly increased incidence and mortality due to SCs of the stomach, liver, pancreas and bladder, as well as increased incidence of cancers of the small intestine and kidney and upper urinary tract, i.e. within the boundaries of the previous RT field as described in available studies.[119,127,177,188-190,204,253-255,268] Although not evident in paper I, a dose-dependency of the abdominal RT field emerged in the Cox regression analysis in paper III, in line with previous publications.[191,253,255] As the use of adjuvant RT was abandoned in Norway during the early 2000s,[15] we expect the malignancies and mortality related to RT to prevail throughout the following decade, before gradually declining.

Increased SIRs and SMRs of soft tissue sarcoma have been reported after PBCT and RT,[195,253-255] however, our results could not confirm these findings. Soft-tissue sarcoma in TCS may reflect transformed teratomas.[269,270]

The combination of alkylating chemotherapy and RT in lymphoma survivors has increased the SC risk in a dose-dependent and additive manner.[271-273] However, the combination of PBCT and RT is not well examined in TCS. In line with one previous study,[169] we observed the highest risks of total SC incidence (SIR 2.14) and non-TC SC mortality (SMR 3.24) following the combination of PBCT and RT.

Increased risk of leukemia was observed after PBCT+RT (paper I) and increased leukemia mortality was observed after PBCT (paper III), although based on very few cases. Increased leukemia mortality was also reported in the Dutch study.[255] Acute myeloid leukemia (AML) is a rare and fatal treatment-related complication associated with both platinum compounds and topoisomerase II inhibitors (e.g. etoposide).[179,274,275] Leukemia associated with cytotoxic agents like PBCT is usually preceded by myelodysplastic syndrome (MDS), involves numerous and complex genetic aberrations and is associated with a poor prognosis.[187,274] The post-treatment latency of leukemia after agents like PBCT is often 2-10 years,[187,274] and this is consistent with the significant increase in leukemia incidence after PBCT+RT within the first 10 years of follow-up. However, two of the three leukemia deaths after PBCT occurred >15 years after the TC treatment. Leukemia associated with etoposide is, on the other hand, usually not preceded by MDS, has a short latency period, often involves one major genetic abnormality of crucial genes, and is associated with a more favorable prognosis.[187,274] PBCT has been associated with leukemia in a dose-dependent manner in TCS.[179] In a recent report, Morton et al. investigated the risk of MDS or AML in 700612 survivors of first primary solid cancer diagnosed 2000-2013, among which 8052 TCS.[276] In line with the expanding use of PBCT to improve survival of many solid cancers during the last two decades, the authors observed increased diagnoses of MDS or AML following cancers previously not associated with leukemia, concluding with a leukemogenicity of PBCT.[276] After TC, they reported a significant 12% excess risk of MDS or AML compared with the general population. The leukemia risk following RT is less clear,[274,276] however the combination of PBCT and RT is associated with higher risk,[119,276] consistent with our results (Paper I).

Corroborating available studies,[127,195,253,255] PBCT was associated with an excess risk of lung cancer incidence (SIR 2.04) and mortality (SMR 1.65). Additionally, in contrast with available

studies,[127,169,188,253,254] abdominal RT was associated with increased lung cancer incidence. Excessive mortality due to cancer of the esophagus was observed after PBCT and mortality due to cancers of the lip/oral cavity/pharynx was increased after both PBCT and RT. Smoking has been associated with higher number and severity of long-term adverse health outcomes in TCS.[277] Smoking-related cancers (lung, bladder, oral cavity/pharynx and esophagus) were associated with as much as 45% of the total SC mortality in a recent report.[256] Smoking has been found to interact with alkylating CT and RT in an additive manner in survivors of Hodgkin`s disease.[271,272] A recent Danish investigation involving 2395 long-term TCS, reported that prevalence of current smoking and overweight were higher in TCS compared with a reference population, and the smoking prevalence among those previously treated with BEP was particularly high.[230] Lifestyle risk factors might reduce the risk of cancer recurrence and enhance prognosis.[278]

Risk of total SC incidence and non-TC SC mortality was significantly increased for seminoma histology compared with nonseminoma with >10 years follow-up in age-adjusted Cox regression analyses. However, in the multivariable models including treatment, histology was not associated with a difference in mortality risk. In our opinion, this demonstrates that it is dissimilarity in treatment and age at diagnosis that results in the differences in risk between seminoma and nonseminoma in crude and relative analyses.

Platinum levels can be detected in plasma for up to 20 years after TC treatment, and is thus a constant source for damage to DNA.[104] It is hypothesized that cytotoxic therapy, especially during childhood and early adulthood, induces cellular senescence, resulting in an early ageing phenotype, increasing the risk of premature adverse health conditions, like development of SCs and increased overall mortality risk.[279-282] Adverse health behaviors after the TC diagnosis may further contribute to the process of accelerated ageing, and thus avoidance of lifestyle stressors may possibly reduce cellular senescence after cytotoxic treatment.[282] We hypothesize that previous TC treatment with PBCT and/or RT, possibly in combination with epigenetic, genetic and lifestyle factors, is the most important risk factor for the increased SC incidence and mortality observed in papers I and III.

5.2.3 Non-cancer mortality (Paper III)

We observed an overall 15% excess risk of non-cancer mortality compared with the expected mortality in a comparable general population and an AER of 4.71, in line with available

reports.[201,255] Compared with the general population, the overall non-TC mortality risk has been described as increasing with time since TC diagnosis,[255] consistent with the observed risks in our study. Fosså et al. indicated that non-cancer mortality did not decline with increasing follow-up time in their study involving 38907 1-year TCS, however the median follow-up time was only 10 years [201] Accordingly, sufficient follow-up time is crucial also when non-cancer mortality in TCS is studied.

Corroborating previous studies,[201,202] we did not observe an increased overall non-cancer mortality risk after surgery compared with the general population. Nonetheless, a significantly increased risk of mortality due to infections emerged after surgery in our study, supported by one previous study,[201] while the other study observed an increased mortality due to infectious disease after CBCT.[127] However, both studies included pneumonia in the category of infectious disease, and although this is clinically relevant, we followed the European Shortlist for causes of death in which pneumonia is grouped together with respiratory diseases.[209]. Kier et al. described that human immunodeficiency virus was the major cause of increased mortality due to infections.[127] In our study, however, acquired immunodeficiency syndrome was the cause of death for only 3 of in total 15 deaths due to infections.

In line with one previous study,[201] we observed an overall 23% excess risk of non-cancer mortality following PBCT. The AER was 4.95 per 10000. The estimates were a bit lower than the SMR of 1.60 and AER of 14.29 reported by a SEER-based study including 15006 nonseminona patients and the SMR of 1.70 reported by a Dutch multicenter study including 6042 TCS, however both studies included information on first-line treatment only.[202,255]

We observed a 65% excess suicide risk compared with general population rates after PBCT. Although the AER was only 1.39, any such avoidable death is devastating. Suicide is more frequent in men than women, and in Norway the median age at suicide is 47 years.[283] In Norway, suicide is the cause of 30-50% of the total mortality in men 20-35 years,[210] and according to the NCoDR, the suicide rates in Norway are similar with suicide rates in Europe, Northern America and Australia.[283] From the literature, TCS disturbingly seems to have an increased suicide risk compared with the general population.[165,204,284] However, studies from Holland and Denmark,[127,255] and the study by Fosså et al.,[201] report no such increased risk. In a review on psychological distress in TCS including 36 studies, anxiety was identified as more common in TCS compared with the general population.[285] Fear of recurrence was also more prevalent, whereas depression and distress were

not. Further, passive coping strategies and treatment-related adverse effects in TCS were associated with an inferior psychological outcome.[285] Removal of a testicle may lead to feelings of loss, uneasiness and shame, and these negative feelings were more common in younger compared with older TCS.[286] Further, a negative change of perceived body image (i.e. reduced masculinity) was reported by 17% in a study of long-term TCS.[153] Additional treatment beyond orchiectomy was associated with significantly increased use of mental health services post-TC treatment in a recent population-based study from Canada.[287] In our study we observed an increased mortality in those <20 years at TC diagnosis, particularly those treated with PBCT. As the majority of those treated with chemotherapy have nonseminoma histology, and because nonseminoma patients are younger at diagnosis than those with seminoma, the increased risk of suicide after chemotherapy also reflects young age at diagnosis. This is in line with the US study by Alanee et al. including 23381 TCS diagnosed 1995-2008, reporting highest suicide rates for those <30 years at TC diagnosis. Taken together, we believe the increased suicide risk after PBCT observed in our study is in part related to bothersome long-term effects of cisplatin together with increased anxiety, negative coping strategies and having received cancer treatment at a vulnerable young age. The results from this study calls for an increased awareness of mental health issues in TCS.

PBCT has been associated with an increased long-term risk of CVD,[111,172,288] presumably through a combination of direct endothelial damage and indirectly through development of risk factors associated with CVD.[8,171] PBCT+RT was observed as especially harmful.[111] Possible genetic pathways associated with CVD risk in TCS treated with PBCT have been identified in genome-wide association studies.[289] We did not find an association between PBCT and increased risk of long-term overall CVD mortality, nor from ischemic heart disease. This is line with some previous reports,[200,202,290] but in contrast with others.[172,201,255] We believe the lack of an association between PBCT and CVD mortality in our study can be explained by the following; Firstly, that a reduction in modifiable CVD risk factors combined with improved treatment options for coronary heart disease has led to a general decline of mortality due to coronary heart disease during the last two decades.[229,291] Secondly, that screening for CVD risk factors was gradually included in the TC follow-up guidelines in Norway from 2007,[112] in line with expanding insights concerning an increased CVD morbidity after PBCT.[110]

Two studies have presented significantly increased risk of mortality due to CVD within the first year after TC diagnosis in those treated with PBCT,[172,202] and our results constricted to the first

year after TC diagnosis supports this association, although based on only three cases. The CVD mortality happening shortly after treatment with CBCT is likely caused by acute vascular injury and endothelial dysfunction.[171,292]

In contrast with previous studies,[201,255] we observed a 17% overall excess of non-cancer deaths compared with the general population following previous treatment with RT. After RT, significantly increased risk of mortality due to benign diseases of the digestive system emerged, as previously reported.[201] In a previous study, gastrointestinal morbidity has been recognized as a possible RT-induced late toxicity.[293] The risk of total non-TC mortality and non-cancer mortality was particularly increased after PBCT+RT, in line with previous reports.[127,201]

6 Conclusions

Previous treatment with PBCT and/or RT was associated with an increased risk of non-TC SC incidence and non-TC mortality. The risks increased with increasing follow-up time beyond 10 years after TC diagnosis. Compared with the general population, the highest risks for non-TC SC and non-TC mortality were observed in those diagnosed with TC at a young age, particularly those previously treated with PBCT. Additionally, surgery only was associated with significantly increased risk of SC development; however, mortality risk was not increased after surgery. PBCT at first TC was associated with a reduced second TC risk in a dose-dependent manner, while treatment with RT did not influence the risk.

More specifically, we observed the following:

i. Compared with the general population, the risk of non-TC SC was significantly increased in all four treatment groups (surgery, PBCT, RT, PBCT+RT). The treatment groups were associated with statistically significant risks for different cancers, compared with general population rates. Compared with surgery, ≥2 CBCT cycles were associated with significantly increased SC risks in those with >10 years follow-up, whereas one adjuvant CBCT or carboplatin were not associated with an increased risk. In comparison with surgery, RT doses of ≥20 Gy to first abdominal field were associated with significantly increased SC risk after >10 years of follow-up, and similar risks were observed following abdominal RT treatment regardless of technique (L-field or paraaortic field).

ii. The crude cumulative incidence of metachronous contralateral TC in the TC cohort was lower after first TC treatment with PBCT and PBCT+RT than after surgery and RT. It was also significantly lower for those <30 years at first TC than those ≥30 years. The second TC risk was 13-fold higher than the risk of developing TC in the general population, and it was highest within the first 5 years of follow-up before gradually declining beyond 20 years. First TC histology was not associated with risk of second TC when adjusting for age and treatment at first TC. Compared with surgery, a dose-dependent inverse relationship emerged for number of CBCT cycles, with a decreasing second TC risk for each additional CBCT cycle administered, significantly reduced after ≥3 CBCT cycles. Treatment with RT did not influence the second TC risk.

iii. Overall non-TC mortality was significantly increased after previous treatment with PBCT, RT or PBCT+RT compared with general population rates, but not after treatment with surgery. Mortality due to non-TC SCs was the most important cause of death, and the treatment groups were associated with mortality due different cancers. Importantly, increased risk of suicide was observed after PBCT. Seminoma vs. nonseminoma histology was not associated with different risks of non-TC mortality when adjusting for age at diagnosis and total treatment burden. Compared with surgery, ≥3 CBCT cycles were associated with an increased risk of non-TC mortality after >10 years follow-up. There was no indication of an increased mortality risk after 1-2 courses of adjuvant CBCT or carboplatin, however observation time is still short.

7 Implications for the future

Given the unselected nationwide cohort, the unique quality of the National Registries in Norway,[222,223] and the complete information on TC treatment burden, this thesis provides new insight on how a TC diagnosis and TC treatment influence the subsequent risk for SC, metachronous contralateral TC, mortality and causes of death. The knowledge generated in this thesis is valuable for health personnel involved in the follow up of TCS, and for present and future TCS of generalizable populations. It is also potentially valuable for survivors of other cancers treated by similar principles.

Actions to prevent a delayed non-TC SC and second TC diagnosis, and hopefully prevent premature deaths, are imperative. Education of TCS themselves regarding the increased non-TC SC and second TC risk in a tailored manner to enhance health literacy and empowerment of TCS`s self-management seem essential. This approach is underscored by the latency of non-TC SC development, non-TC mortality risk and the long-term second TC risk. In addition to information regarding CVD risk, second TC risk and lifestyle recommendations, information concerning the increased SC risk related to PBCT and RT has recently been included in an updated written patient care plan issued to TCS and the patient´s general practitioner in Norway and Sweden at the end of hospital follow-up.[15] As the second TC risk may persist beyond 20 years, TCS, especially the youngest TCS treated with surgery only, must be well-informed about the importance of a lifelong self-examination of the remaining testicle.

The results in this thesis support a constant evaluation of whether further reduction of cytotoxic treatment burden is possible without deterioration of TC survival. As a consequence of the increasing awareness of the non-TC SC risk associated with PBCT and RT, the recommended treatment of seminoma stage IIA has been modified to a primary unilateral RPLND, with PBCT or RT as possible treatment options, in the newly updated management program of SWENOTECA.[15] Clinical implementation of the promising biomarker miR-371a-3p may hopefully extend the possibility of a more personalized TC treatment in the near future.[59,60]

Our results with increased incidence and mortality due to smoking and alcohol-related cancers, suggests that lifestyle improvements are important in TCS. TCS would presumably benefit from an implementation of counseling on lifestyle improvements in their survivorship care. It is highly recommended that future studies investigating late effects of cytotoxic treatment in TCS

strive to include information regarding modifiable risk factors. The possible cellular senescence brought on by cytotoxic treatment deserves attention, and an evaluation of possible underlying mechanisms and interventions in TCS is highly warranted. Further, future studies investigating biological pathways that influence the risk of non-TC SC and mortality in TCS are needed.

The clinical role of transscrotal ultrasound for detection of GCNIS remains unclear.[294,295] The results of this thesis agrees with the present risk-adapted biopsy strategy of the contralateral testicle.[9] and supports the need for future studies on the role of miR-371a-3p in detection of GCNIS.

The increased risk of suicide after PBCT suggests that assessments regarding the mental health and suicide risk factors of TCS, especially the youngest TCS with additional treatment beyond orchiectomy, is important. Considerations as to whether psychosocial issues should be included in the follow-up of TCS are recommended.

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Appendix I-III

Appendix I

Variables retrieved from medical journals

Hospital

Personal identification number

Date of birth

Registered

Comments

Date of orchiectomy

Bilateral testicular cancer (no/yes synchronous/yes metachronous)

Histology (seminoma/nonseminoma)

Clinical stadium according to Royal Marsden

Surveillance if clinical stage I (no/yes)

Date of first chemotherapy

Stop date chemotherapy

Reason for first-line chemotherapy (adjuvant/metastatic/recurrence)

First chemotherapy regimen

Number of cycles first chemotherapy

Second chemotherapy regimen

Number of cycles second chemotherapy

Third chemotherapy regimen

Number of cycles third chemotherapy

Fourth chemotherapy regimen

Number of cycles fourth chemotherapy

Fifth chemotherapy regimen

Number of cycles fifth chemotherapy

Radiotherapy field (no/field-type)

Dose of radiotherapy in Gray

Retroperitoneal lymph node dissection (no/yes)

Date of first post-orchiectomy surgery

Type of first post-orchiectomy surgery

Histology first post-orchiectomy surgery

Date of second post-orchiectomy surgery

Type of second post-orchiectomy surgery

Histology second post-orchiectomy surgery

Date of third post-orchiectomy surgery

Type of third post-orchiectomy surgery

Histology third post-orchiectomy surgery

Recurrence after primary treatment (yes/no)

Clinical stage at first recurrence

Date of first recurrence

Treatment first recurrence

Second recurrence (yes/no)

Clinical stage at second recurrence

Date of second recurrence

Treatment second recurrence

Dead (yes/no)

Cause of death

Appendix II

European shortlist						
ID	**Code**	**Level**	**Description**	**ICD-10**	**ICD-9**	**ICD-8**
1	1.	1	Infectious and parasitic diseases	A00-B99	001-139	000-136
2	1.1	2	Tuberculosis	A15-A19, B90	010-018, 137	010-019
3	1.2	2	AIDS (HIV disease)	B20-B24	042-044 (279.1)	-
4	1.3	2	Viral hepatitis	B15-B19, B94.2	070	070
5	1.4	2	Other infectious and parasitic diseases	A00-A09, A20-B09, B25-B89, B91-B94.1, B94.8-B99	001-009, 020-041, 045-066, 071-136, 138-139	000-009, 020-068, 071-136
6	2.	1	Neoplasms	C00-D48	140-239	140-239
7	2.1	2	Malignant neoplasms	C00-C97	140-208	140-209
8	2.1.1	3	Malignant neoplasm of lip, oral cavity, pharynx	C00-C14	140-149	140-149
9	2.1.2	3	Malignant neoplasm of oesophagus	C15	150	150
10	2.1.3	3	Malignant neoplasm of stomach	C16	151	151

11	2.1.4	3	Malignant neoplasm of colon, rectum and anus	C18-C21	153-154	153-154
12	2.1.5	3	Malignant neoplasm of liver and intrahepatic bile ducts	C22	155	155, 197.8
13	2.1.6	3	Malignant neoplasm of pancreas	C25	157	157
14	2.1.7	3	Malignant neoplasm of larynx	C32	161	161
15	2.1.8	3	Malignant neoplasm of trachea, bronchus, lung	C33-C34	162	162
16	2.1.9	3	Malignant melanoma of skin	C43	172	172
17	2.1.10	3	Malignant neoplasm of breast	C50	174-175	174
18	2.1.11	3	Malignant neoplasm of cervix uteri	C53	180	180
19	2.1.12	3	Malignant neoplasm of other and unspecified parts of uterus	C54-C55	179, 182	182
20	2.1.13	3	Malignant neoplasm of ovary	C56	183.0	183.0

21	2.1.14	3	Malignant neoplasm of prostate	C61	185	185
22	2.1.15	3	Malignant neoplasm of kidney	C64	189.0	189.0
23	2.1.16	3	Malignant neoplasm of bladder	C67	188	188
24	2.1.17	3	Malignant neoplasm of brain and central nervous system	C70-C72	191-192	191-192
25	2.1.18	3	Malignant neoplasm of thyroid	C73	193	193

26	2.1.19	3	Hodgkin disease and lymphomas	C81-C86	200-201	200-201
27	2.1.20	3	Leukemia	C91-C95	204-208	204-208
28	2.1.21	3	Other malignant neoplasm of lymphoid and hematopoietic tissue	C88, C90, C96	202-203	202-203

29	2.1.22	3	Other malignant neoplasms	C17, C23-C24, C26-C31, C37-C41, C44-C49, C51-C52, C57-C60, C62-C63, C65-C66, C68-C69, C74-C80, C97	152, 156, 158-160, 163-171, 173, 181, 183.2-184, 186-187, 189.1-190, 194-199	152, 156, 158-160, 163-171, 173, 181, 183.1-184, 186-187, 189.1-190, 194-197.7, 197.9-199
30	2.2	2	Non-malignant neoplasms (benign and uncertain)	D00-D48	209-239	210-239

31	3.	1	Diseases of the blood and blood forming organs and certain disorders involving the immune mechanism	D50-D89	280-289	280-289
32	4.	1	Endocrine, nutritional and metabolic diseases	E00-E89	240-279	240-279
33	4.1	2	Diabetes mellitus	E10-E14	250	250

34	4.2	2	Other endocrine, nutritional and metabolic diseases	E00-E07, E15-E89	240-246, 251-279	240-246, 251-279
35	5.	1	Mental and behavioral disorders	F01-F99	290-319	290-315
36	5.1	2	Dementia	F01, F03	290	290
37	5.2	2	Alcohol abuse (including alcoholic psychosis)	F10	291, 303	291, 303
38	5.3	2	Drug dependence, toxicomania	F11-F16, F18-F19	304-305	304-305
39	5.4	2	Other mental and behavioral disorders	F04-F09, F17, F20-F99	292-302, 306-319	292-302, 306-315
40	6.	1	Diseases of the nervous system and the sense organs	G00-H95	320-389	320-389
41	6.1	2	Parkinson’s disease	G20	332.0	342
42	6.2	2	Alzheimer’s disease	G30	331.0	-
43	6.3	2	Other diseases of the nervous system and the sense organs	G00-G12, G14, G21-G25, G31-H95	320-330, 331.1331.9, 332.1-389	320-341, 343-389

44	7.	1	Diseases of the circulatory system	I00-I99	390-459	390-444.1, 444.3-458, 782.4
45	7.1	2	Ischaemic heart diseases	I20-I25	410-414	410-414
46	7.1.1	3	Acute myocardial infarction	I21-I22	410-411	410-411
47	7.1.2	3	Other ischaemic heart diseases	I20, I23-I25	412-414	412-414
48	7.2	2	Other heart diseases	I30-I51	420-429	420-429
49	7.3	2	Cerebrovascular diseases	I60-I69	430-438	430-438
50	7.4	2	Other diseases of the circulatory system	I00-I15, I26-I28, I70-I99	390-405, 415-417, 440-459	390-404, 440-444.1, 444.3-458, 782.4
51	8.	1	Diseases of the respiratory system	J00-J99	460-519	460-519
52	8.1	2	Influenza	J09-J11	487	470-474
53	8.2	2	Pneumonia	J12-J18	480-486	480-486
54	8.3	2	Chronic lower respiratory diseases	J40-J47	490-494, 496	491-493, 518
55	8.3.1	3	Asthma	J45-J46	493	493

56	8.3.2	3	Other chronic lower respiratory diseases	J40-J44, J47	490-492, 494, 496	491-492, 518
57	8.4	2	Other diseases of the respiratory system	J00-J06, J20-J39, J60-J99	460-478, 495, 500519	460-466, 490, 500-517, 519
58	9.	1	Diseases of the digestive system	K00-K92	520-579	520-577, 444.2
59	9.1	2	Ulcer of stomach, duodenum and jejunum	K25-K28	531-534	531-534
60	9.2	2	Cirrhosis, fibrosis and chronic hepatitis	K70, K73-K74	571	571
61	9.3	2	Other diseases of the digestive system	K00-K22, K29-K66, K71-K72, K75-K92	520-530, 535-570, 572-579	520-530, 535-570, 572-577, 444.2
62	10.	1	Diseases of the skin and subcutaneous tissue	L00-L99	680-709	680-709
63	11.	1	Diseases of the musculoskeletal system/connective tissue	M00-M99	710-739	710-738
64	11.1	2	Rheumatoid arthritis and osteoarthrosis	M05-M06, M15-M19	714-715	712-713

65	11.2	2	Other diseases of the musculoskeletal system/connective tissue	M00-M02, M08-M13, M20-M99	710-712, 716-739	710-711, 714-738
66	12.	1	Diseases of the genitourinary system	N00-N99	580-629	580-629, 792
67	12.1	2	Diseases of kidney and ureter	N00-N29	580-594	580-594
68	12.2	2	Other diseases of the genitourinary system	N30-N99	595-629	595-629, 792
69	13.	1	Complications of pregnancy, childbirth and puerperium	O00-O99	630-676	630-678
70	14.	1	Certain conditions originating in the perinatal period	P00-P96	760-779	760-779
71	15.	1	Congenital malformations and chromosomal abnormalities	Q00-Q99	740-759	740-759
72	16.	1	Symptoms, signs, ill-defined causes	R00-R99	780-799	780-782.3, 782.5-791, 793-796
73	16.1	2	Sudden infant death syndrome	R95	798.0	-

74	16.2	2	Unknown and unspecified causes	R96-R99	798.1-9, 799.0,2-3,59	795-796

75	16.3	2	Other symptoms, signs, ill-defined causes	R00-R94	780-797, 799.1, 799.4	780-782.3, 782.5-791, 793-794
76	17.	1	External causes of morbidity and mortality	V01-Y89	E800-E999	E800-E999
77	17.1	2	Accidents	V01-X59, Y85-Y86	E800-E929	E800-E929, E940-E946
78	17.1.1	3	Transport accidents	V01-V99, Y85	E800-E848, E929.0-1	E800-E845, E940-E941
79	17.1.2	3	Accidental falls	W00-W19	E880-E888	E880-E887
80	17.1.3	3	Drowning and accidental submersion	W65-W74	E910	E910
81	17.1.4	3	Accidental poisoning	X40-X49	E850-E869	E850-E877
82	17.1.5	3	Other accidents	W20-W64, W75- X39, X50-59, Y86	E870-E879, E890-E909, E911-928, E929.2-9	E890-E909, E911-929, E942-E946
83	17.2	2	Suicide and intentional self-harm	X60-X84, Y87.0	E950-E959	E950-E959
84	17.3	2	Homicide, assault	X85-Y09, Y87.1	E960-E969	E960-E969

85	17.4	2	Events of undetermined intent	Y10-Y34, Y87.2	E980-E989	E980-E989
86	17.5	2	Other external causes of injury and poisoning	Y35-Y84, Y88-Y89	E930-E949, E970-E978, E990-E999	E930-E936, E943-E949, E970-E978, E990-E999

Appendix III

Informasjonsskriv

Forskningsprosjekt: Ny kreftsykdom og dødelighet hos menn som er behandlet for testikkelkreft

Jeg er prosjektleder for et nasjonalt forskningsprosjekt der vi ønsker å kartlegge alvorlige senfølger etter tidligere kreftbehandling ved testikkelkreft. Vi planlegger å inkludere informasjon om alle menn behandlet for testikkelkreft i Norge i perioden 1980 til og med 2009. Informasjon om sykdomsutbredelse og behandling vil bli hentet fra pasientjournalene. Vi vil hente data fra Kreftregisteret for å se på antallet som har fått en ny kreftdiagnose i perioden etter behandlingen for testikkelkreft, samt data fra Dødsårsaksregisteret for å kartlegge dødsårsaker for de som er døde. Dette er et prosjekt som vil kunne hjelpe oss med å bedre oppfølgingen etter avsluttet behandling for testikkelkreft.

Prosjektet innebærer ikke noen ekstra kontroller eller legebesøk for ditt vedkommende. Vi vil ikke avdekke informasjon om deg som du ikke allerede er kjent med. Data om deg vil bli lagret avidentifisert på en forskningsserver på Universitetssykehuset i Nord-Norge. Forskningsresultatene vil etter hvert bli offentliggjort gjennom publisering i internasjonale, anerkjente tidsskrifter.

Etisk Komité i Region Sør-øst har godkjent dette prosjektet. Det er ikke krav om aktivt samtykke for deltagelse i prosjektet. Det er imidlertid stilt som vilkår for prosjektet at alle gis muligheten til å reservere seg mot deltagelse i prosjektet. Det innebærer at alle aktuelle pasienter vil bli inkludert i studien med mindre en selv aktivt gir beskjed om at en ikke ønsker å delta. Det betyr at om du har motforestillinger mot å delta i studien, så må du selv kontakte undertegnede som er prosjektansvarlig.

Vennlig hilsen

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Papers I-III

Paper I

IJC
International Journal of Cancer

Continuing increased risk of second cancer in long-term testicular cancer survivors after treatment in the cisplatin era

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Using complete information on total treatment burden, this population-based study aimed to investigate second cancer (SC) risk in testicular cancer survivors (TCS) treated in the cisplatin era. The Cancer Registry of Norway identified 5,625 1-year TCS diagnosed 1980–2009. Standardized incidence ratios (SIRs) were calculated to evaluate the total and site-specific incidence of SC compared to the general population. Cox regression analyses evaluated the effect of treatment on the risk of SC. After a median observation time of 16.6 years, 572 TCS developed 651 nongerm cell SCs. The SC risk was increased after surgery only (SIR 1.28), with site-specific increased risks of thyroid cancer (SIR 4.95) and melanoma (SIR 1.94). After chemotherapy (CT), we observed 2.0- to 3.7-fold increased risks for cancers of the small intestine, bladder, kidney and lung. There was a 1.6- to 2.1-fold increased risk of SC after ≥2 cycles of cisplatin-based CT. Radiotherapy (RT) was associated with 1.5- to 4.4-fold increased risks for cancers of the stomach, small intestine, liver, pancreas, lung, kidney and bladder. After combined CT and RT, increased risks emerged for hematological malignancies (SIR 3.23). TCS treated in the cisplatin era have an increased risk of developing SC, in particular after treatment with cisplatin-based CT and/or RT.

Introduction

Patients with germ cell testicular cancer (TC) have a 15-year relative survival rate exceeding 98% in Norway.[1] An important factor for the excellent prognosis was the introduction of cisplatin in the late 1970s.[2,3] However, the relative overall survival beyond 20 years after successful TC treatment is continuously decreasing.[4] One explanation is second cancer (SC) development which is a severe and possibly life-threatening late effect after cancer treatment.[5]

Previous studies have demonstrated a 1.7 to 3.5-fold increased risk for both hematological and solid nongerm cell SC in testicular cancer survivors (TCS) compared to age-matched general populations.[6–9] The risk has been associated with both radiotherapy (RT) and chemotherapy (CT), but not with surgery only. The

Additional Supporting Information may be found in the online version of this article.

Key words: testicular cancer, second cancer, survivorship, cancer epidemiology, radiotherapy, chemotherapy, surgery, germ cell

Abbreviations: CBCT: cisplatin-based chemotherapy; CRN: Cancer Registry of Norway; CT: chemotherapy; HR: hazard ratio; IQR: interquartile range; RPLND: retroperitoneal lymph node dissection; RT: radiotherapy; SC: second cancer; SIR: standardized incidence ratio; TC: testicular cancer; TCS: testicular cancer survivors

Conflict of interest: The authors declare no potential conflicts of interest.

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What's new?

Long-term survival to 15 years among germ cell testicular cancer survivors treated in the cisplatin era, marked by the introduction of cisplatin in the late 1970s, generally has been excellent. Beyond 20 years, however, survival rates decline. In this analysis of data on Norwegian men diagnosed with testicular cancer between 1980 and 2009, an increased overall risk for nongerm cell second cancer was detected among survivors, despite treatment. Risk was elevated in particular beyond 10 years of follow-up after cisplatin-based chemotherapy or radiotherapy. Despite reduced treatment intensity, two or more cycles of cisplatin-based chemotherapy was associated with continuing increased second cancer risk.

majority of these studies have, however, been based on outdated TC treatment principles. Consequently, there is a lack of studies on SC risk after the introduction of cisplatin.[9–12] Experimental data and animal studies have suggested cisplatin as a carcinogen.[13] Besides, high cumulative cisplatin doses have been linked to an increased leukemia risk.[14,15]

Three recent publications have evaluated SC risk after cisplatin-based chemotherapy (CBCT) in TCS, demonstrating a 40–80% excess risk.[7–9] However, two of these studies lack complete treatment information.[7,9] Rather than calculating standardized incidence ratios (SIRs), Kier *et al*. calculated the cumulative incidence of SC and hazard ratios (HR) by using a control group from the general population matched 10:1 on age at diagnosis.[8] Importantly, this study presented favorable results for the surveillance group, demonstrating no excess risk of SC or reduced survival compared to the control group.

The aim of this population-based study was to investigate the risk of nongerm cell SC among TCS in the cisplatin era, by (*i*) comparing the incidence of SC to that of the general population, and (*ii*) investigating the risks associated with different treatment modalities (surgery, RT, CT and the surveillance strategy).

Methods

Study cohort and design

Men diagnosed with histologically verified germ cell TC from January 1, 1980, to December 31, 2009, were identified through the Cancer Registry of Norway (CRN).[1] Major exclusion criteria included extragonadal germ cell cancer, a prior malignancy, age <16 years at TC diagnosis and death or SC before 12 months follow-up (Supporting Information Fig. S1). Follow-up started 12 months after diagnosis to avoid inclusion of synchronous or treatment-unrelated cancer.

The final study cohort consisted of 5,625 one year survivors of first primary germ cell TC. Detailed information regarding disease stage, histology and primary and subsequent TC treatment was abstracted from medical records and linked with CRN data on subsequent cancer diagnoses, updated through December 31, 2016.

This historical prospective cohort study was approved by the Regional Committee for Medical and Health Research Ethics and the Data Protection Authorities at the University Hospital of North Norway. All eligible TCS still alive have received a study information letter with the possibility to withdraw from participation (passive consent). Twenty-three men (0.38%) declined participation, for reasons undisclosed.

Staging and treatment groups

The clinical staging of TC was based on the Royal Marsden Hospital staging system.[16] Overall, treatment intensity has gradually been reduced during the study period in line with increasing knowledge about efficacy and toxicity (Supporting Information Table S1).[2,17] The number of CT cycles used to treat patients with initially metastatic disease have been reduced over the years from ≥4 to 3 cycles for patients with good prognosis (the majority of patients) and 4 cycles for patients with intermediate and poor prognosis.[2,18] During the study period, the usage of RT for stage I seminoma and primary retroperitoneal lymph node dissection (RPLND) for early stages of nonseminoma was gradually abandoned (Supporting Information Table S1).

The study cohort was categorized into three groups by decade of TC diagnosis. It was further categorized into treatment groups by overall treatment burden: Surgery only (including surveillance, n = 1,394; 25%), CT (n = 2,471; 44%), RT (n = 1,542; 27%) and CT and RT combined (CT + RT; n = 218; 3.9%; Table 1).

Statistical methods

Categorical variables are presented with numbers and percent, while continuous variables are presented with median and interquartile range (IQR), unless otherwise stated.

Participants were followed from the time of their first TC + 1 year, until the development of a nongerm cell SC of interest, death, emigration or December 31, 2016, whichever occurred first. To avoid immortal time bias (a period of follow-up during which, by design, the outcome of interest cannot occur), treatment was analyzed as a time-varying covariate. For instance, a patient accrued person-years of observation time in the surgery only group until the date they received CT or RT.

The crude probability of SC was estimated by the cumulative incidence using the Aalen-Johansen estimator,[19] treating death from any cause as a competing risk.

SIRs were calculated to evaluate the total and site-specific incidence of SC in the TC cohort compared to the general population. A subgroup analysis was performed for those initially designated to surveillance. SIRs were obtained by dividing the observed number of cancers in the cohort by the expected number in a TC-free, male Norwegian population, matched by 5-year age groups and calendar year of follow-up. SIRs were calculated for the total cohort and for different treatment groups, taking the time-varying treatment exposure into account. Results are presented with

Table 1. Patient characteristics according to the decade of first primary TC diagnosis

	Decade of first primary TC diagnosis			
	1980–1989 (*n* = 1,274)	**1990–1999 (*n* = 1,896)**	**2000–2009 (*n* = 2,455)**	**All (*n* = 5,625)**
Treatment, *n* (%)				
Surgery only[1]	244 (19)	359 (19)	791 (32)	1,394 (25)
CT	413 (32)	735 (39)	1,323 (54)	2,471 (44)
RT[2]	518 (41)	729 (38)	295 (12)	1,542 (27)
CT + RT	99 (7.8)	73 (3.9)	46 (1.9)	218 (3.9)
Age at diagnosis, years	31.9 (26.2–39.8)	32.5 (26.7–40.0)	33.8 (27.9–41.4)	32.9 (27.1–40.7)
Seminoma	36.3 (30.1–44.9)	36.4 (30.7–44.4)	37.2 (31.6–44.6)	36.7 (30.8–44.5)
Nonseminoma	27.9 (23.3–33.9)	28.7 (23.9–34.9)	29.6 (24.8–36.4)	28.8 (24.2–35.3)
Age at diagnosis, *n* (%)				
‹20 years	77 (6.0)	82 (4.3)	59 (2.4)	218 (3.9)
20–30 years	468 (37)	671 (35)	764 (31)	1,903 (34)
30–40 years	417 (33)	663 (35)	926 (38)	2,006 (36)
40–50 years	187 (14)	298 (16)	474 (19)	959 (17)
›50 years	125 (10)	182 (10)	232 (10)	539 (9.6)
Histology, *n* (%)				
Seminoma	619 (49)	967 (51)	1,356 (55)	2,942 (52)
Nonseminoma	655 (51)	929 (49)	1,099 (45)	2,683 (48)
Observation time, years	29.3 (24.2–32.2)	20.5 (18.0–23.5)	11.3 (8.8–14.0)	16.6 (10.9–23.8)
Observation time, *n* (%)				
‹10 years	99 (7.8)	132 (7.0)	959 (39)	1,191 (21)
10–19 years	128 (10)	712 (38)	1,496 (61)	2,336 (42)
20–29 years	480 (38)	1,052 (55)	0	1,532 (27)
30–37 years	567 (44)	0	0	567 (10)
Initial disease stage, *n* (%)[3]				
I	798 (63)	1,348 (71)	1829 (74)	3,975 (71)
Mk+/II	325 (25)	359 (19)	440 (18)	1,124 (20)
III	31 (2.4)	43 (2.3)	40 (1.6)	114 (2.0)
IV	120 (9.4)	146 (7.7)	146 (6.0)	412 (7.3)
Cause of first-line CT, *n* (%)				
Adjuvant, CSI	39 (7.6)	199 (25)	639 (47)	877 (32)
Primary metastatic disease	410 (80)	513 (63)	601 (44)	1,524 (57)
Recurrence	63 (12)	96 (12)	129 (9.4)	288 (11)
First CT regimen, *n* (%)				
BEP-20	129 (25)	552 (68)	839 (61)	1,520 (57)
CVB	324 (63)	36 (4.5)	0	360 (13)
EP	6 (1.2)	36 (4.5)	208 (15)	250 (9.3)
Other CBCT[4]	44 (8.6)	118 (15)	21 (1.5)	183 (6.8)
Adjuvant carboplatin	1[5] (0.2)	26 (3.2)	287 (21)	314 (12)
CEB	3 (0.6)	31 (3.8)	8 (0.6)	42 (1.6)
Other[6]	5 (1.0)	9 (1.1)	6 (0.4)	20 (0.7)
CBCT cycles, *n* (%)[7]				
1	8 (1.6)	30 (4.0)	188 (17)	226 (10)
2	27 (5.3)	116 (15)	177 (16)	320 (14)
3	93 (18)	106 (14)	252 (24)	451 (19)
4	289 (57)	351 (47)	381 (35)	1,021 (43)
›4	90 (18)	149 (20)	84 (7.8)	323 (14)

(Continues)

Table 1. Patient characteristics according to the decade of first primary TC diagnosis (Continued)

	Decade of first primary TC diagnosis			
	1980–1989 (*n* = 1,274)	1990–1999 (*n* = 1,896)	2000–2009 (*n* = 2,455)	All (*n* = 5,625)
CBCT containing vinca alkaloids or etoposide, *n* (%)				
Vinca alkaloids	257 (50)	61 (7.6)	0	318 (12)
Etoposide	153 (30)	649 (80)	1,080 (79)	1882 (70)
Both	98 (19)	66 (8.2)	10 (0.7)	174 (6.5)
Other CT	4 (0.8)	32 (4.0)	279 (20)	315 (12)
RT first field, *n* (%)				
L-field[8]	549 (89)	626 (78)	224 (66)	1,399 (80)
Paraaortic	24 (3.9)	147 (18)	99 (29)	270 (15)
Supradiaphragmatic	7 (1.3)	5 (0.6)	1 (0.3)	13 (0.7)
Supra- and infradiaphragmatic[9]	21 (3.4)	0	0	21 (1.2)
RT metastatic[10]	16 (2.6)	24 (3.0)	17 (5.0)	57 (3.2)
RT dose for first field, Gy	36.0 (36.0–40.0)	30.0 (25.2–30.0)	25.2 (25.2–30.0)	30.0 (27.0–36.0)
RT dose for first field[11]				
20–29 Gy	7 (1.1)	309 (38)	208 (60)	524 (30)
30–39 Gy	409 (66)	462 (58)	125 (36)	996 (56)
≥40 Gy	199 (32)	24 (3.0)	10 (2.9)	233 (13)
Total recurrences, *n* (%)	99 (7.8)	166 (8.8)	206 (8.4)	471 (8.4)
Initial surveillance, *n* (%)[12]	75 (5.9)	387 (20)	911 (37)	1,373 (24)
Recurrences in initial surveillance group, *n* (%)[13]	19 (25)	72 (19)	122 (13)	213 (16)

Note: Data are presented as median (IQR), unless otherwise stated.
Abbreviations: BEP-20, bleomycin, etoposide and cisplatin; CBCT, cisplatin-based CT; CEB, carboplatin, etoposide and bleomycin; CSI, clinical stage I; CT + RT, combination of CT and RT; CT, chemotherapy; CVB, cisplatin, vinblastine and bleomycin; EP, etoposide and cisplatin; Gy, grey; IQR, interquartile range; Mk+, marker positive; *n*, number; RT, radiotherapy; TC, testicular cancer.
[1]The surgery only group included men followed with surveillance after orchiectomy (*n* = 1,146; 20%) and men submitted to additional retroperitoneal lymph node dissection without CT or RT (*n* = 248; 4.4%).
[2]There were a total of 10 individuals that received scrotal RT of 16–20 Gy because of carcinoma *in situ* or a new tumor of the remaining testicle who underwent partial orchiectomy. These 10 individuals are not included in the RT group in our analyses.
[3]As described by Peckham *et al*. Combined management of malignant teratoma of the testis.[16]
[4]Of which a total of 139 were dose-escalated CBCT.
[5]Adjuvant carboplatin administered in 2005 because of metachronous TC.
[6]Constitutes the following regimes: carboplatin monotherapy in metastatic setting (*n* = 16), sendoxan/adriamycin (*n* = 1), CAOS (actinomycin D, adriamycin, vincristine, sendoxan; *n* = 2), actinomycin D (*n* = 1).
[7]Number of total CBCT cycles administered. May have received additional CT regimens, but these are not accounted for in this number.
[8]L-field or dogleg-field. Included in this category are also 52 individuals who received RT of groin in addition to L-field and 9 individuals who received a reversed Y-field.
[9]Sixteen of 21 individuals received infradiaphragmatic RT as first RT field and a short while later received supradiaphragmatic RT.
[10]RT toward bone (*n* = 19), CNS (*n* = 16), abdominal residual masses (*n* = 16), intraoperative RT (*n* = 1), skin lesions (*n* = 1) and nonspecified sites (*n* = 4).
[11]Overall, 17 TCS for various reasons received only 1–20 Gy (2, 9 and 6 TCS from first to last decade, respectively). One patient received versions of overlapping infradiaphragmatic fields two times within 3 years. For this, one case the dose presented is an addition of Field 1 and Field 2.
[12]This group consists of all cases with CSI initially intended for surveillance as treatment strategy.
[13]The percentage stated is the amount of recurrences among those initially treated with surveillance.

observed numbers of SC in our database, SIRs and 95% confidence intervals (95% CIs).

The effect of treatment was analyzed in age-adjusted Cox regression models with follow-up time as time scale and the surgery only group as a reference. The proportional hazard assumption for the analysis of treatment groups was judged to be violated using both visual inspection of −log−log survival curves and a significant Schoenfeld test ($p = 0.005$). All analyses were thus performed using a time-dependent Cox model with two-way interaction terms between each treatment and a dummy variable of follow-up time (before/after 10 years). Similar subgroup analyses were performed to evaluate the SC risk in relation to histology and treatment intensity. When we investigated the association between the number of CBCT cycles and risk of SC, men who had subsequently received RT were censored at the start date for their first RT treatment. Likewise, when analyzing effects of the first RT field and abdominal RT dose, individuals who had received CT were censored at the date of administration of CT. Estimates are presented for those with >10 years observation time, starting 1 year from TC diagnosis, unless otherwise specified. Results are presented as HRs with corresponding 95% CIs.

Data were analyzed using Stata statistical software (version MP 14.2; STATA, College Station, TX). A p-value <0.05 was considered significant.

Table 2. SIRs for nongerm cell SC according to treatment group

	Total			Surgery only[1]			CT			RT			CT + RT		
	n[2]	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI
Total SC	572	**1.58**	**1.45–1.71**	96	**1.28**	**1.05–1.56**	174	**1.62**	**1.39–1.88**	270	**1.64**	**1.46–1.85**	32	**2.14**	**1.51–3.02**
All solid cancers C00–C80	529	**1.44**	**1.32–1.57**	88	1.16	0.94–1.43	161	**1.52**	**1.30–1.77**	252	**1.49**	**1.31–1.68**	28	**1.81**	**1.25–2.63**
Ear, nose and throat C00–14, C31–32	19	1.16	0.74–1.81	3	0.92	0.30–2.85	7	1.44	0.69–3.02	9	7.60	0.62–2.28	0	0	0
Esophagus C15	8	1.50	0.75–3.00	2	1.87	0.47–7.47	4	2.61	0.98–6.94	2	0.80	0.20–3.18	0	0	0
Stomach C16	21	**2.19**	**1.43–3.36**	2	1.05	0.26–4.19	1	0.39	0.06–2.79	12	**2.56**	**1.45–4.51**	6	**12.98**	**5.83–28.90**
Small intestine C17	11	**4.29**	**2.38–7.74**	2	3.74	0.93–14.93	3	**3.73**	**1.20–11.56**	5	**4.43**	**1.84–10.63**	1	**10.48**	**1.48–74.4**
Colorectal C18–20	69	**1.27**	**1.01–1.61**	11	1.01	0.56–1.82	22	1.46	0.96–2.22	34	1.32	0.94–1.84	2	0.86	0.21–3.43
Liver and bile ducts C22, C24	12	**2.11**	**1.20–3.72**	2	1.70	0.42–6.79	1	0.58	0.08–4.13	8	**3.13**	**1.56–6.26**	1	4.49	0.63–31.85
Pancreas C25	28	**2.77**	**1.92–4.02**	4	1.98	0.74–5.27	3	1.09	0.35–3.37	19	**3.90**	**2.46–6.11**	2	**4.54**	**1.14–18.16**
Lung C34	67	**1.54**	**1.21–1.96**	8	0.95	0.48–1.90	23	**2.04**	**1.35–3.07**	32	**1.47**	**1.04–2.08**	4	2.01	0.76–5.37
Skin, malignant melanoma C43[3]	42	**1.49**	**1.07–1.96**	12	**1.94**	**1.10–3.42**	18	**1.86**	**1.17–2.95**	11	0.91	0.50–1.64	1	0.93	0.13–6.63
Skin, other C44	24	1.46	0.98–2.17	3	0.88	0.28–2.72	6	1.39	0.63–3.10	13	1.63	0.94–2.80	2	2.69	0.67–10.77
Soft tissue C47–C49	6	**2.33**	**1.04–5.17**	1	1.80	0.25–12.81	1	1.14	0.16–8.08	3	2.85	0.92–8.84	1	**10.51**	**1.48–74.61**
Prostate C61	122	1.08	0.90–1.29	23	1.02	0.68–1.53	33	1.08	0.78–1.52	63	1.14	0.88–1.46	3	0.64	0.21–1.99
Kidney and upper urinary tract C64–C66	37	**1.94**	**1.41–2.68**	3	0.76	0.25–2.36	13	**2.22**	**1.29–3.83**	19	**2.23**	**1.42–3.50**	2	2.70	0.68–10.80
Bladder C67	57	**2.25**	**1.73–2.91**	4	0.78	0.29–2.09	20	**2.97**	**1.91–4.60**	30	**2.42**	**1.69–3.46**	3	2.66	0.86–8.25
Brain C70–C72, C75.1	28	1.24	0.86–1.80	7	1.42	0.68–2.98	12	1.50	0.85–2.65	9	1.02	0.53–1.96	0	0	0
Thyroid C73[4]	10	**2.81**	**1.51–5.22**	4	**4.95**	**1.86–13.18**	2	1.5	0.36–6.00	3	2.31	0.75–7.16	1	**8.51**	**1.20–60.42**
Malignant neoplasm of other and ill-defined sites C76	10	**2.02**	**1.09–3.75**	1	1.03	0.14–7.30	4	3.30	1.24–8.79	5	1.99	0.83–4.78	0	0	0
All hematological malignancies C81–C85, C88, C90–C93, C95, D45, D46	53	**1.31**	**1.00–1.71**	9	1.05	0.55–2.02	15	1.18	0.71–1.95	24	1.36	0.91–2.02	5	**3.23**	**1.35–7.77**
Lymphoma C81–C85	27	1.31	0.90–1.91	6	1.36	0.61–3.04	5	0.74	0.30–1.77	13	1.50	0.87–2.59	3	**3.96**	**1.28–12.29**
Leukemia C91–C93, C95	15	1.43	0.86–2.38	1	0.46	0.06–3.25	5	1.55	0.65–3.72	7	1.51	0.72–3.18	2	**4.86**	**1.22–19.44**

Notes: Significant results marked with bold. SIRs reported for cancers or groups of cancers with occurrence of ≥5. The following SC were observed in the dataset, but not included in analysis: malignant neoplasm of other and ill-defined digestive organs (C26; *n* = 2), malignant neoplasm of bone and articular cartilage (41, *n* = 3), mesothelioma (C45; *n* = 4), male breast cancer (C50; *n* = 2), penis (C60; *n* = 2) and eye (C69; *n* = 1). Significant results marked with bold. C refers to diagnostic code according to the ICD-10 classification.

Abbreviations: 95% CI, 95% confidence interval; CT + RT, combination of chemotherapy and radiotherapy; CT, chemotherapy; IQR, interquartile range; *n*, number; RT, radiotherapy; SC, nongerm cell second cancer; SIR, standardized incidence ratio.

[1]Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.

[2]Observed number in cohort. For total SC, *n* represents total cases diagnosed with SC in the cohort. For site-specific analyses, *n* represents the occurrence of the diagnosis of interest in the cohort.

[3]Overall, median time to melanoma diagnosis was 14.6 years (IQR 7.2–17.8).

[4]Overall, median time to thyroid cancer diagnosis was 5.8 years (IQR 2.5–18.5).

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Table 3. SIRs for nongerm cell SC by age at first treatment, follow-up time and attained age at first SC diagnosis, according to treatment group

	Total			Surgery only[1]			CT			RT			CT + RT		
	n[2]	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI	*n*	SIR	95% CI
Total SC	572	**1.58**	**1.45–1.71**	96	**1.28**	**1.05–1.56**	174	**1.62**	**1.39–1.88**	270	**1.64**	**1.46–1.85**	32	**2.14**	**1.51–3.02**
Age at first treatment															
<20 years	7	**2.29**	**1.09–4.80**	0	NA	NA	6	**3.17**	**1.43–7.06**	0	NA	NA	1	**8.00**	**1.13–56.77**
20–30 years	88	**1.95**	**1.58–2.41**	18	**1.69**	**1.06–2.68**	36	**1.76**	**1.27–2.44**	28	**2.27**	**1.56–3.28**	6	**3.75**	**1.69–8.35**
30–40 years	164	**1.65**	**1.41–1.92**	19	0.96	0.62–1.51	53	**1.73**	**1.32–2.27**	84	**1.86**	**1.50–2.30**	8	1.97	0.99–3.94
40–50 years	155	**1.55**	**1.33–1.82**	28	**1.74**	**1.20–2.52**	39	**1.44**	**1.05–1.97**	75	**1.44**	**1.15–1.80**	13	**2.95**	**1.71–5.08**
>50 years	157	**1.39**	**1.19–1.63**	30	1.15	0.81–1.65	40	**1.45**	**1.07–1.98**	83	**1.52**	**1.23–1.88**	4	0.83	0.31–2.21
Follow-up time															
<10 years	141	**1.28**	**1.09–1.51**	43	**1.52**	**1.13–2.05**	48	1.28	0.97–1.70	42	1.03	0.76–1.39	8	**2.38**	**1.19–4.77**
10–20 years	217	**1.58**	**1.39–1.81**	30	1.16	0.81–1.66	56	**1.48**	**1.14–1.92**	122	**1.80**	**1.51–2.15**	9	1.58	0.82–3.04
20–30 years	175	**1.81**	**1.56–2.09**	19	1.10	0.70–1.73	56	**2.11**	**1.62–2.74**	87	**1.81**	**1.46–2.23**	13	**2.59**	**1.50–4.46**
30–37 years	39	**2.12**	**1.55–2.90**	4	1.04	0.39–2.78	14	**2.41**	**1.43–4.08**	19	**2.43**	**1.55–3.81**	2	2.12	0.53–8.47
Attained age at first SC diagnosis															
<40 years	31	**1.65**	**1.16–2.35**	11	**2.16**	**1.19–3.89**	13	1.41	0.82–2.42	6	1.52	0.68–3.38	1	2.28	0.32–16.19
40–60 years	244	**1.59**	**1.40–1.80**	40	1.27	0.93–1.73	91	**1.68**	**1.37–2.07**	98	**1.56**	**1.28–1.90**	15	**2.71**	**1.63–4.49**
60–75 years	236	**1.55**	**1.36–1.76**	37	1.26	0.92–1.74	54	**1.45**	**1.11–1.90**	130	**1.64**	**1.38–1.95**	15	**2.18**	**1.31–3.61**
75–90 years	61	**1.64**	**1.28–2.11**	8	0.87	0.44–1.74	16	**2.27**	**1.39–3.71**	36	**1.91**	**1.38–2.65**	1	0.47	0.07–3.33

Note: Significant results marked with bold.
Abbreviations: 95% CI, 95% confidence interval; CT + RT, combination of chemotherapy and radiotherapy; CT, chemotherapy; *n*, number; RT, radiotherapy; SC, nongerm cell second cancer; SIR, standardized incidence ratio.
[1]Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.
[2]Observed number. For total SC, *n* represents total cases diagnosed with SC in the cohort.

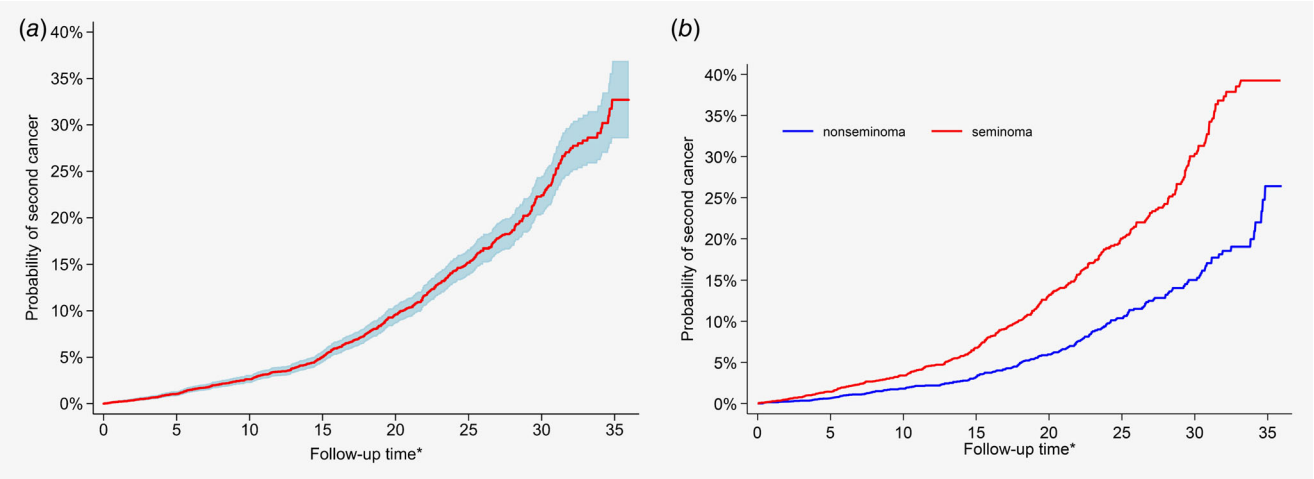

Figure 1. Crude cumulative probability of second cancer by follow up-time. (*a*) All patients (with 95% confidence interval) and (*b*) by histology. In *a*, the red line indicates the probability of second cancer, and the blue area indicates the 95% confidence interval. *years since diagnosis +1 year. [Correction added on 1 May 2020, after first online publication: Figure 1b was incorrect due to a mathematical error and has been replaced in this version.]

Data availability

The data that support the outcomes of our study are available from the CRN (SC) and a local database (treatment information). Restrictions apply to the availability of these data, which were used under license for our study. Data can be requested by application to the CRN.

Results

Study cohort

Over the decades, the use of surgery only or CT increased, while there was decreasing use of RT or CT + RT (Table 1). Median age at diagnosis was 32.9 years (IQR 27.1–40.7), 36.7 years for seminomas and 28.8 years for nonseminomas. Median observation time for the total cohort was 16.6 years (IQR 10.9–23.8), and 37% had an observation time >20 years.

From 1980–1989 to 2000–2009, the proportion of chemotherapy-treated men receiving adjuvant CT for stage I disease increased from 7.6% to 47%, and the use of the surveillance strategy increased from 5.9% to 37% (Table 1). Of the 1,373 (24%) men subjected to surveillance, 213 (16%) experienced a recurrence.

Overall and site-specific risk of SC in TCS compared to the general population

Overall, 572 TCS (10.2%) developed 651 SCs, with prostate, lung, bladder, melanoma and colon cancer being the most common malignancies (Supporting Information Table S2).

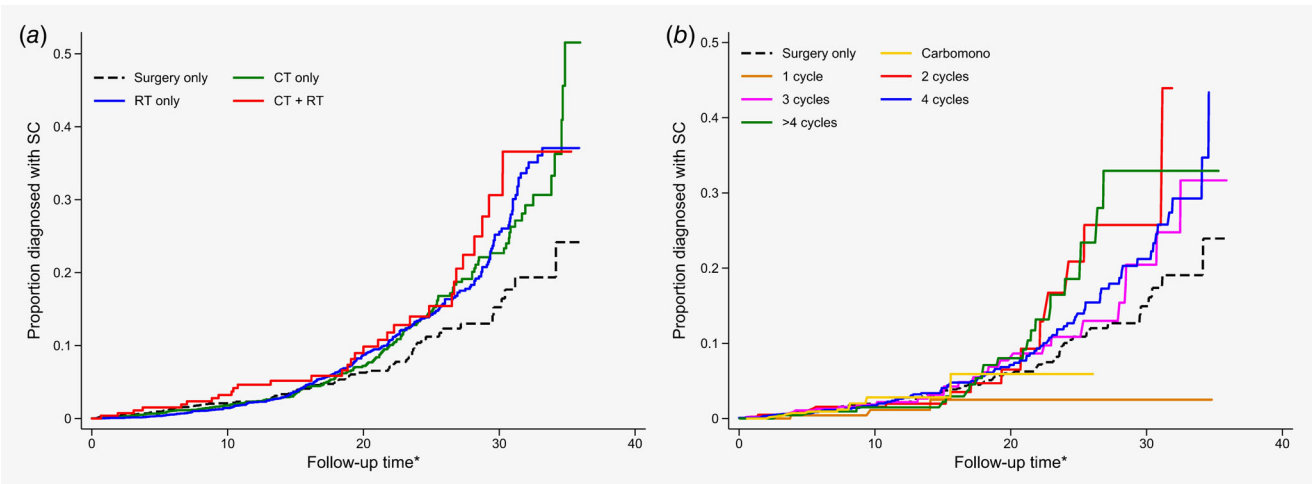

Figure 2. Proportion diagnosed with second cancer by follow-up time, adjusted for age at testicular cancer diagnosis. (*a*) By treatment, (*b*) by number of cisplatin-based chemotherapy cycles and carboplatin monotherapy. *years since diagnosis +1 year. Abbreviations: Carbomono, adjuvant carboplatin monotherapy; CT + RT, combination of CT and RT; CT, chemotherapy; RT, radiotherapy; SC, second cancer.

The crude probability of SC accelerated beyond 15–20 years (2.6% at 10 years and 15.2% at 25 years for the total cohort; Fig. 1*a*).

The TCS had a 58% overall excess risk of developing nongerm cell SC (SIR 1.58, 95% CI 1.45–1.71) compared to the general population. All treatment groups had significantly increased risks, ranging from 28% excess risk after surgery only to twofold increased risk after CT + RT (Table 2).

The overall excess risk of developing a solid cancer was 44%, with significantly elevated risks for cancers of the stomach, small intestine, colon/rectum, liver/bile ducts, pancreas, lung, melanoma, soft tissue, kidney, bladder and thyroid. In addition, the TCS had an overall increased risk of hematological malignancies (SIR 1.31, 95% CI 1.00–1.71).

After surgery only, there were increased risks for melanoma (SIR 1.94, 95% CI 1.10–3.42) and cancer of the thyroid (SIR 4.95, 95% CI 1.86–13.18; Table 2). CT was associated with a significantly 1.9 to 3.7-fold increased risk of cancers of the small intestine, lung, melanoma, kidney and bladder. After RT, the risks were 1.5–4.4 times significantly increased for cancers of the stomach, small intestine, liver and bile ducts, pancreas, lung, kidney and bladder. CT + RT increased the risks for cancers of the stomach, small intestine, pancreas, soft tissue, thyroid, lymphoma and leukemia (Table 2).

Table 4. HRs for total and solid nongerm cell SC according to treatment intensity

	Total SC		Solid SC	
	HR	**95% CI**	**HR**	**95% CI**
CBCT cycles[1]				
Surgery only	1	ref	1	ref
1	0.41	0.07–2.54	0.47	0.07–2.92
2	**1.91**	**1.01–3.59**	**2.19**	**1.16–4.15**
3	1.41	0.83–2.37	1.24	0.70–2.21
4	**1.60**	**1.12–2.30**	**1.73**	**1.19–2.50**
>4	**2.09**	**1.23–3.53**	**2.19**	**1.27–3.78**
Carboplatin[2]	1.17	0.18–7.68	2.54	0.62–10.43
Other[3]	2.21	0.80–6.11	1.77	0.55–5.71
Vinca alkaloids *vs*. etoposide				
Surgery only	1	ref	1	ref
Vinca alkaloids	**1.64**	**1.09–2.48**	**1.82**	**1.19–2.77**
Etoposide	**1.56**	**1.07–2.26**	**1.57**	**1.06–2.32**
Both vinca alkaloids and etoposide	**1.79**	**1.02–3.13**	**1.84**	**1.03–3.29**
Other CT	0.55	0.08–4.02	1.22	0.30–5.03
RT field				
Surgery only	1	ref	1	ref
L-field[4]	**1.66**	**1.23–2.25**	**1.76**	**1.29–2.42**
Paraaortic	1.65	0.95–2.87	1.73	0.97–3.06
Other[5]	**4.40**	**1.07–18.07**	**5.06**	**1.23–20.85**
RT dose for first abdominal RT field				
Surgery only	1	ref	1	ref
20–29 Gy	**1.88**	**1.21–2.90**	**2.01**	**1.28–3.16**
30–39 Gy	**1.71**	**1.25–2.33**	**1.80**	**1.30–2.51**
≥40 Gy	1.42	0.93–2.18	1.50	0.96–2.33

Notes: Significant results marked with bold. Results presented for patients with >10 years observation time. Results for hematological SCs not shown as none were significant.

Abbreviations: 95% CI, 95% confidence interval; CBCT, cisplatin-based chemotherapy; CT, chemotherapy; Gy, grey; HR, hazard ratio; RT, radiotherapy; SC, second cancer.

[1]Number of total CBCT cycles administered. May have received additional CT regimens, but these are not accounted for in this number. A total of 140 TCS received dose-escalated CBCT, of which 1, 27, 12, 35 and 65 men received 1, 2, 3, 4 or >4 cycles, respectively. Then, 13% of those that received dose-escalated CBCT developed SC, compared to 7% in the CT-group overall and 9% in the CT-group when excluding those that received adjuvant CT.

[2]Carboplatin monotherapy, carboplatin in adjuvant setting for stage I seminoma.

[3]Thirty-three CEB (carboplatin, etoposide, bleomycin; of which 32 received 4 cycles and 1 received 2 cycles of CEB), 4 other carboplatin-based CT (3 of which received 4 cycles and 1 received 1 cycle) and 1 actinomycin D.

[4]L-field and variations: The majority received L-field or dogleg-field. Included in this category are also 52 cases who received RT of groin in addition to L-field and 9 cases who received a reverse Y-field.

[5]Eleven supra- and infradiaphragmatic fields, two RT in metastatic setting (bone and abdominal residual tumor).

In TCS initially intended for surveillance, the SIR was 1.34, 95% CI 1.07–1.68, with a significantly increased risk for thyroid cancer (SIR 7.35, 95% CI 3.06–17.66).

Both seminoma and nonseminoma histology were associated with increased risks of SC with SIRs 1.59 (95% CI 1.44–1.76) and 1.55 (95% CI 1.35–1.77), respectively.

Risk of SC by age and follow-up time in TCS compared to the general population

The risk of SC generally declined with increasing age at initial treatment for TC, regardless of which treatment was given. Overall, SIRs ranged from 2.29 (95% CI 1.09–4.80) among patients who initiated treatment before 20 years of age to 1.39 (95% CI 1.19–1.63) among those 50 years or older (Table 3).

The risk of SC generally increased with increasing follow-up time. Overall, SIRs ranged from 1.28 (95% CI 1.09–1.51) among TCS followed less than 10 years to 2.12 (95% CI 1.55–2.90) among patients followed for 30–37 years. Significantly increased risks of SC after CT or RT alone did only emerge with follow-up beyond 10 years, while significantly increased SC risk after surgery was only present with less than 10 years of follow-up.

Overall, SIRs were relatively similar at 1.6 regardless of attained age at first SC diagnosis. Unlike the other treatment groups, the increased SC risk among patients who received surgery only was restricted to SC diagnosed before 40 years of age.

Overall and site-specific risk of SC by histology and treatment group compared to surgery only

The crude cumulative probability of SC at 25 years was 20% (95% CI 18–22%) for seminoma and 10% (95% CI 8.7–12%) for nonseminoma survivors (Fig. 1*b*). SC risk among individuals with seminoma was significantly increased compared to nonseminoma in age-adjusted analysis (HR 1.20, 95% CI 1.01–1.44). [Correction added on 1 May 2020, after first online publication: The values in the preceding paragraph have been corrected.]

With surgery only as the reference group, SC risks increased with observation time in all treatment groups (Fig. 2*a*, Supporting Information Table S3), except among the 11 nonseminoma patients treated with RT only when stratifying according to histology (Supporting Information Fig. S2). Risks of solid SCs were significantly increased >10 years of follow-up regardless of treatment group, with HRs ranging from 1.65 to 1.79. The only significantly increased SC risk <10 years of follow-up was for all hematological malignancies after CT + RT (HR 8.73, 95% CI 1.76–43.29).

Compared to the surgery group, we observed a significant 5.1 to 5.3-fold excess risk of bladder cancer after CT or RT, a 7.6-fold excess risk of kidney cancer after RT, and a 24-fold excess risk of cancer of the stomach after combined CT + RT.

SC risk in relation to treatment intensity

The time to development of SC by number of CBCT cycles is illustrated in Figure 2*b*. After >10 years of follow-up, we observed a 1.6 to 2.1-fold excess risk of SC after two or more CBCT cycles compared to surgery only (Table 4). Similar excess risk was found for solid cancer, but not for hematological cancer. No increased SC risk was observed after one CBCT cycle or adjuvant carboplatin, however median observation time was only 9.5 years.

Both the L-field technique and paraaortic RT were associated with 1.6-fold increased risks for SC in comparison to surgery only (Table 4). After paraaortic RT, 9.3% developed SC, of which 0.4% (n = 1) was bladder cancer, compared to 19% developing SC after L-field, of which 1.7% (n = 22) were bladder cancers. SC risks were also increased after RT doses of ≥20 Gy to the first abdominal field.

Discussion

In this national TCS cohort treated since 1980, we found, to the best of our knowledge for the first time, a significantly increased overall risk for nongerm cell SC among TCS treated with surgery only when compared to the general population, with site-specific excess risks of thyroid cancer and melanoma. We also demonstrated that contemporary treatment with CBCT leads to a continuing increased risk of SC, with significantly increased site-specific risk of cancers of the small intestine, lung, melanoma, kidney and bladder. Two or more cycles of CBCT were associated with an excess risk of SC, and CT in combination with RT led to particularly high risks.

The considerable latency from cancer therapy to SC occurrence, as well as the excess risk with increasing follow-up time in our study cohort, is comparable to previous findings,[7–9,20] and underscores the importance of designing studies with sufficient observation time when investigating SC risk in cancer survivors.

Previous publications have reported an excess risk of thyroid cancer after CBCT[7,9] or RT.[20] The elevated risk of thyroid cancer in the surgery only group reported herein, although based on relatively few cases, is a novel finding that needs to be further elucidated in future research. The median time to development of thyroid cancer in our study population was 5.8 years, and our findings may partly be explained by surveillance bias. A few rare inherited syndromes that can cause both thyroid and testicular tumors have been described however,[21] and thyroid cancer can on rare occasions develop from teratomas.[22] It is unknown whether this was the case in our study population.

Excess risk of melanoma in TCS after RT has been reported in previous studies,[20,23,24] but in line with results reported by van den Belt-Dusebout *et al.*,[25] we demonstrated a significant excess risk of melanoma in the surgery only group. However, the number of cases diagnosed with melanoma was low, even though our study includes hitherto the highest number of patients with complete treatment details. Some authors have attributed these findings to increased medical attention during the first years of follow-up.[23] Surveillance bias is a less likely explanation in our cohort due to the long median latency of 14.6 years between diagnosis of TC and melanoma.

Patients with cutaneous melanoma have been found to be at increased risk of developing SC, including testicular and thyroid cancer.[26] There is a genetic link between thyroid cancer and melanoma through a susceptibility to BRAF mutations. A 2014 US

study found a reciprocal twofold increased risk of developing papillary thyroid cancer after cutaneous melanoma or *vice versa*, and a high incidence of BRAF v600e-mutations.[27] In our study population, no patients presented with both thyroid cancer and melanoma.

An association between childhood tumor risk and first-degree family history of solid tumors was recently observed for several solid cancers, including melanomas, even after controlling for probable hereditary cancer syndromes.[28] The increased risk of SC after surgery only, together with the young age at TC diagnosis and the familial risk of developing TC, similarly implies a genetic susceptibility and/or that environmental factors during fetal life or early childhood predispose for both TC and other malignancies.[29–31] The genetic susceptibility for TC is thought to be driven by multiple low-penetrance alleles.[32–34] Additionally, a recent study demonstrated evidence for CHEK2 as a moderate-penetrance susceptibility gene.[35] To this date, however, TC has not been linked to a cancer syndrome that predisposes to other cancers,[32] but our findings suggest that further research within this field should be prioritized. CT-scans during follow-up after treatment for TC have been associated with increased SC risk,[36,37] and might contribute to the excess risk in the surgery only group. Future studies evaluating the impact of follow-up with CT-scans *vs*. MRI should be prioritized.

The increased overall SC risk after surgery alone only before 10 years of follow-up could indicate surveillance bias (Table 3), even though follow-up started 1 year after TC diagnosis. However, in that case, we would also expect increased SC risks after RT or CT before 10 years of follow-up, which was not seen. In summary, we believe that our findings in general are not explained by surveillance bias.

In line with previous publications, we demonstrated a 62% increased risk of SC after treatment with CT in the cisplatin era.[7–9] Bladder cancer was among the most frequent SCs in our study cohort, corroborating previous reports,[7–9,20,25] and we observed a threefold increased risk for bladder cancer after CT when compared to the general population. The risks for cancers of the kidney and upper urinary tract and lung were twofold increased following CT, which is comparable to previous reports.[7–9] There is a possibility that at least some of the cancers diagnosed as soft tissue sarcoma are in fact transformed teratomas,[38,39] but we did not find any increased risk of sarcomas after CBCT as previously reported.[7,9]

Cisplatin is a platinum compound which has been detected in plasma decades after treatment,[40] and in most organs several months after treatment,[41,42] where it remains partly reactive. Despite the lack of long-term data, the accumulation of platinum might be a pathophysiological explanation for the increased risk of SC.[10] In a recent publication by Hjelle *et al*., a reduced risk of SC was found in individuals with larger long-term declines in serum-platinum levels.[43] Importantly, platinum is eliminated through renal clearance, and it has been detected in urine up to 16 years after treatment.[44] An association between CBCT and cancers of the urinary tract is therefore likely.

The 64% excess SC risk following RT confirms the established association between RT and subsequent SC development.[8,9,20,25] The increased risks of cancers of the gastrointestinal tract, pancreas, liver, lung, kidney and bladder after RT compared to the general population reported herein, are in line with previous publications demonstrating that SCs often are localized in relation to previous RT fields.[20,45–48] The excess risk was almost similar after both paraaortic lymph node portal and the more extensive L-field portal, which also includes ipsilateral iliac lymph nodes. The association was, however, not statistically significant after paraaortic RT, probably due to the low number and the shorter follow-up. The absolute numbers suggested that the risk of developing bladder cancer was reduced after paraaortic RT compared to L-field, but statistical analysis was not possible because of low numbers. We could not confirm a linear trend for increasing risk of solid SC with increasing abdominal RT dose, as reported by Groot *et al*.,[9] despite our larger study population.

In our study, combined CT and RT was associated with the highest risks for SC compared to the general population, which is in agreement with previous reports.[49–51] The increased risk of stomach cancer after combination therapy has been previously reported.[25] The risks for all hematological malignancies, lymphoma and leukemia were also increased after CT + RT. Subsequent hematological malignancies generally develop within 10 years following cancer treatment,[14,52] and our results were consistent with this.

To the best of our knowledge, analyses of TCS intended for surveillance after surgery has not been performed previously, and also in this group, we found a significantly increased risk of SC. Kier *et al*. presented favorable results for the surveillance group,[8] however these authors' findings were based on a group that excluded all individuals that relapsed from analyses. There is an ongoing debate as to whether surveillance is superior to adjuvant chemotherapy in the treatment of stage I TC. Of note, we did not observe any increased risk of SC after one cycle of CBCT or carboplatin, but the observation time is still short, and longer follow-up is needed before any conclusions can be drawn.

We found an almost 60% significantly increased risk of SC after both seminomas and nonseminomas compared to the general population, which is in line with the recent Dutch publication.[9] Our remarkably higher 25-year crude probability of all SCs following seminomas of 20%, compared to 12.6% in the Dutch report is interesting. [Correction added on 1 May 2002, after first online publication: 28% has been changed to 20% in the preceding sentence.] Some of the difference might be explained by the longer median follow-up after seminoma in our study of 16.0 years compared to 13.5 years in the Dutch study.

Strengths of our study are the inclusion of detailed information regarding total treatment burden for the entire study cohort, and the unique quality of the CRN. Based on a distinct personal identification number used in Norway, the CRN receives information from several sources to ensure accuracy, and reporting to

this registry is instructed by law.[1] SIRs are easy to understand and interpret, and we considered that calculation of absolute excess risks (AERs) would not provide more information to the reader. The use of time-dependent Cox-regression implements the important element of observation time in our analyses.

Limitations include the lack of details regarding known risk factors for cancer, for example, smoking, hereditary factors and comorbidities. There is, however, no reason to believe that smoking prevalence among TCS differs from the general population.[53,54]

In conclusion, despite reduced treatment intensity during the last decades, we find a continuing increased risk of SC in TCS treated in the cisplatin era. While treatment-related late effects remain the main culprit, increased SC risks among patients treated with surgery only suggest that genetic and environmental factors are also important. Regardless of cause, improvement of lifestyle behavior, in particular, smoking cessation, reduction of alcohol intake, increased physical activity and a healthy diet may reduce the risk of SC.[55] Promotion and guidance for a healthy lifestyle should thus be implemented to a larger degree during long-term follow-up of all TCS than it is today. Health care professionals must be aware of the SC risk so that proper examination is initiated by the slightest suspicion of a SC to ensure diagnosis at an early stage.

Disclaimer

The study has used data from the Cancer Registry of Norway. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway is intended nor should be inferred.

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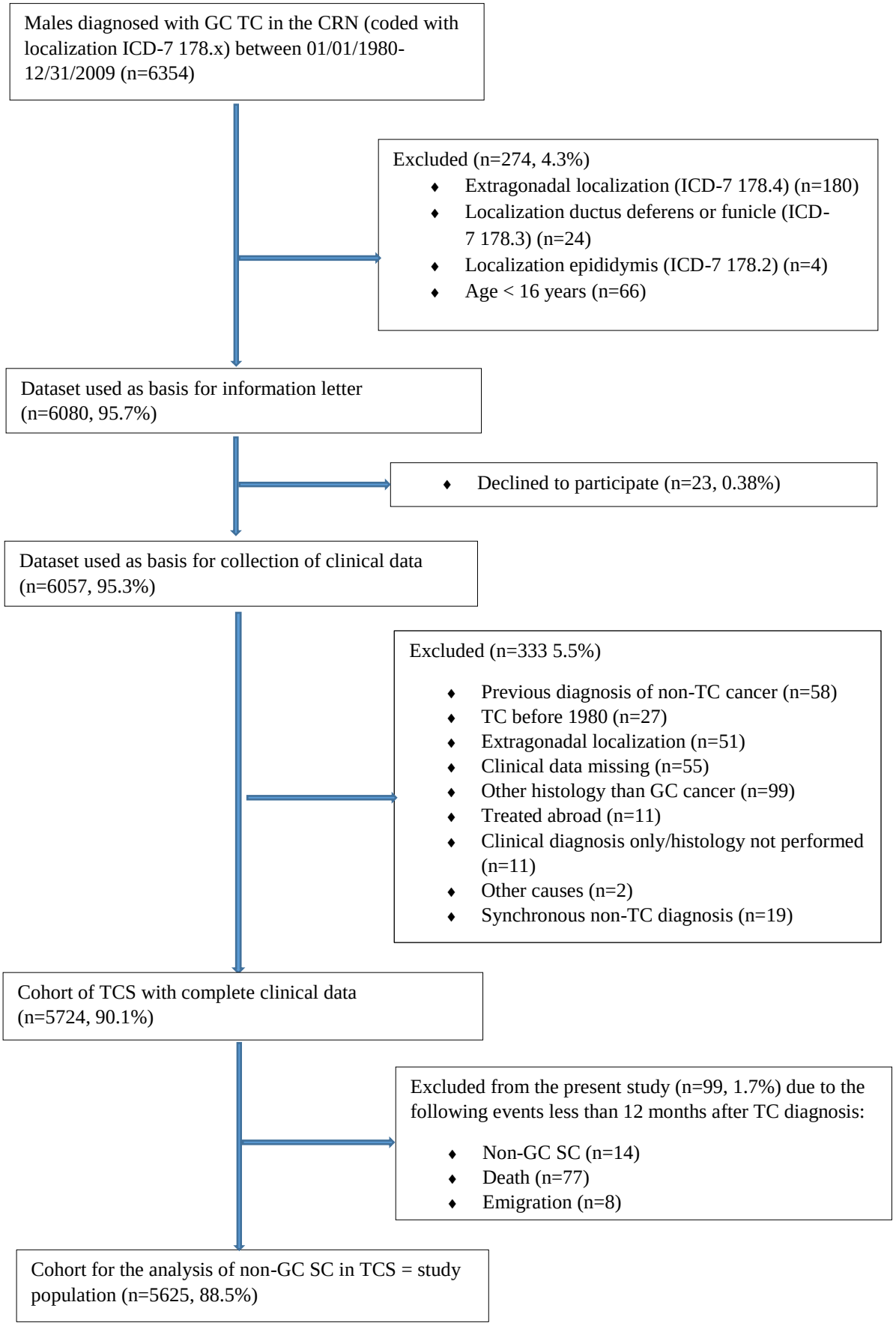
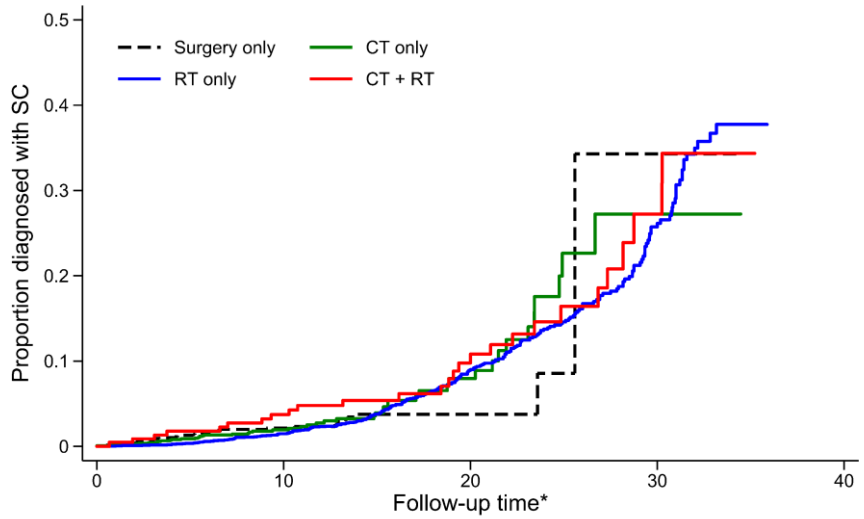


Supplemental appendix Figure 1. Flow chart presenting the study cohort

Abbreviations: GC: germ cell; TC: testicular cancer; CRN: Cancer Registry of Norway; ICD-7: International Classification of Diseases version 7; SC: second cancer; TCS: testicular cancer survivors.

A)



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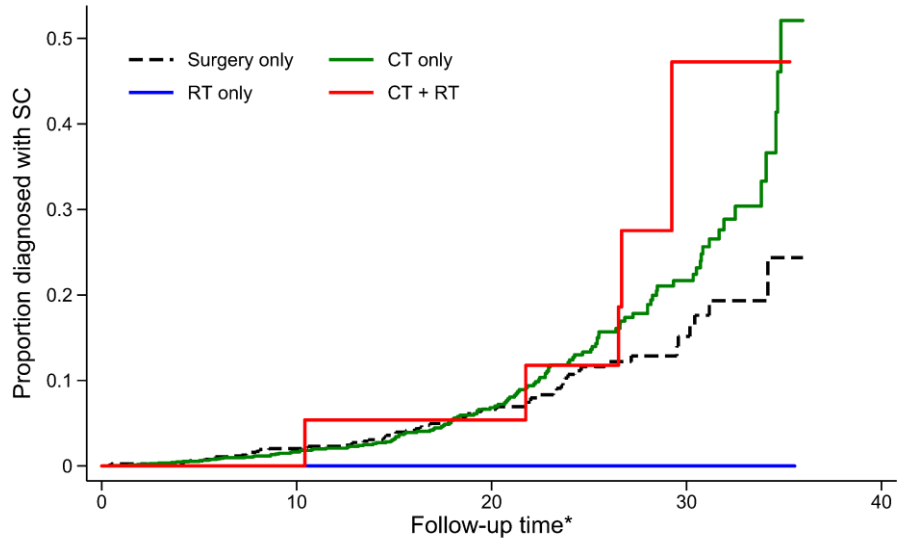

Supplemental Appendix Figure 2. Proportion diagnosed with second cancer after seminoma or nonseminoma by follow up-time, adjusted for age at testicular cancer diagnosis. A) Seminoma, B) Nonseminoma.

*years since diagnosis + 1 year

Abbreviations: SC: second cancer; CT: chemotherapy; RT: radiotherapy; CT + RT: combination of CT and RT.

Supplemental appendix Table 1. General treatment principles for TC patients in Norway by decade of diagnosis

Decade	Localized disease	Metastatic disease
1980 to 1989	Seminomas: adjuvant RT towards paraaortal and ipsilateral iliacal lymph nodes by the L-field technique.[1] The target dose was gradually reduced from 36-40 Gy to 25.2-27 Gy.[1, 2] One institution offered RT restricted to the para-aortic area only from 1989.[3] Nonseminomas: staging RPLND followed by adjuvant chemotherapy if metastases were histologically verified.[4]	Majority of cases treated with CT. CVB standard CT-regimen up until 1987 when BEP became standard treatment.[5] Some treated according to experimental regimens within research protocols.[6-11] Generally ≥4 cycles administered. Seminoma patients received post-chemo RT to residual masses until 1986. Residual masses after CT in nonseminoma patients were resected, primarily as a RPLND. RT was a treatment option if residual masses persisted after CT and/or surgery. Nerve-sparing RPLND from 1989.[4]
1990 to 1999	Seminomas: adjuvant RT continued as above, target dose usually <30 Gy. Nonseminomas: After 1990, primary RPLND was abandoned, and stage 1 patients were instead offered surveillance or 1-2 cycles of adjuvant CBCT.[12-14]	The BEP-regimen remains standard first-line therapy in metastatic disease. High-dose chemotherapy with autologous stem cell support available from 1995. Some treated according to experimental regimens within research protocols.[6-11] Residual masses after CT in nonseminoma patients were resected, primarily as a RPLND
2000 to 2009	Seminomas: From 2000, RT was gradually abandoned in stage I, and patients were increasingly offered surveillance or adjuvant carboplatin.[15, 16] Nonseminomas: patients are offered surveillance or one adjuvant cycle of BEP.[17] Follow-up: By the end of the study-period recommendation to use MRI-scan because of the concern about increased second cancer risk after multiple CT-scans.[18, 19]	The number of CT cycles have been reduced to 3 cycles for patients with good prognosis (the majority of patients) and 4 cycles for patients with intermediate and poor prognosis.[11, 20] Seminoma patients offered EP instead of BEP. Decrease in usage of RT for seminomas, but still an option in stage IIA disease.

TC: testicular cancer; RT: radiotherapy; RPLND: retroperitoneal lymph node dissection; CT: chemotherapy; CVB: cisplatin, vinblastine, bleomycin; BEP: cisplatin, etoposide, bleomycin; Gy: Grey; CBCT: cisplatin-based CT; MRI: magnetic resonance imaging.

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Supplemental appendix Table 2. Presentation of numbers of first and subsequent non-germ cell SC in the study cohort according to diagnostic code

Diagnostic code ICD-10	First	Second	Third	Fourth	SUM
C00-C14 Ear, nose, throat	14	1	0	0	15
C15 Esophagus	8	0	0	0	8
C16 Stomach	20	1	0	0	21
C17 Small intestine	8	3	0	0	11
C18 Colon	35	6	0	0	41
C19 Rectosigmoid junction	4	0	0	0	4
C20 Rectum	24	2	0	0	26
C22 Liver and intrahepatic bile ducts	6	0	0	0	6
C24 Extrahepatic bile ducts	6	0	0	0	6
C25 Pancreas	25	3	0	0	28
C26 Ill-defined digestive organs	1	1	0	0	2
C31 Accessory sinuses	1	0	0	0	1
C32 Larynx	3	1	0	0	4
C34 Bronchus and lung	65	3	1	0	69
C41 Bone and articular cartilage	2	0	1	0	3
C43 Malignant melanoma of skin	38	6	1	0	45
C44 Other malignant neoplasms of skin	22	2	1	0	25
C45 Mesothelioma	4	0	0	0	4
C47 Peripheral nerves and autnomic nervous system	1	0	0	1	2
C48 Retroperitoneum and peritoneum	2	0	0	0	2
C49 Other connective and soft tissue	2	0	0	0	2
C50 Breast	2	0	0	0	2
C60 Penis	2	0	0	0	2
C61 Prostate	107	11	2	2	122
C64 Kidney	23	4	0	0	27
C65 Renal pelvis	2	1	0	0	3
C66 Ureter	4	3	2	0	9
C67 Bladder	49	7	1	0	57
C68 Other and unspecified urinary organs	0	1	0	0	1
C69 Eye	1	0	0	0	1
C70 Meninges	4	0	0	0	4
C71 Brain	12	0	0	0	12
C72 Spinal cord, cranial nerves and other parts of CNS	3	0	0	0	3
C75.1 Pituitary gland	9	0	0	0	9
C73 Thyroid	10	0	0	0	10
C76 Other and ill-defined sites	8	2	0	0	10
C81 Hodgkin lymphoma	7	0	0	0	7
C82 Follicular lymphoma	8	0	1	1	10
C83 Non-follicular lymphoma	6	1	1	0	8
C85 Non-Hodgkin lymphoma, unspecified	2	1	0	0	3
C88 Malignant immunoproliferative diseases	1	0	0	0	1
C90 Multiple myeloma	3	1	0	0	4
C91 Lymphoid leukaemia	5	0	0	0	5
C92 Myeloid leukaemia	6	1	1	0	8
C93 Monocytic leukaemia	1	0	0	0	1
C95 Leukaemia, unspecified	1	0	0	0	1
D45 Polycytemia vera	1	0	0	0	1
D46 Myelodysplastic syndrome	4	1	0	0	5
SUM	**572**	**64**	**12**	**4**	**651**

Note: Data are presented as numbers. Thirteen cases are registered with identical ICD-10 diagnoses twice, and as a result, the sum in this table does not add up to the numbers presented in table 2 for certain diagnoses. Median time between first and second diagnosis: 2.04 years (IQR 4.75); median time between second and third diagnosis: 1.54 years (IQR 4.05); median time between third and fourth diagnosis: 0.33 years (IQR 0.34).

SC: non-germ cell second cancer; CNS: central nervous system; ICD-10: international classification of diseases; IQR: interquartile range.

Supplemental Table 3. HRs for non-germ cell SC according to treatment group: age-adjusted time-dependent Cox

	Surgery only[1]		CT		RT		CT + RT	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Total SC[2]								
>10 y obs	1	ref	**1.57**	**1.13-2.16**	**1.71**	**1.27-2.31**	**1.71**	**1.06-2.78**
>15 y obs[3]	1	ref	**1.78**	**1.23-2.60**	**1.83**	**1.29-2.62**	**1.85**	**1.07-3.19**
>20 y obs	1	ref	**1.96**	**1.22-3.14**	**1.78**	**1.13-2.80**	**2.08**	**1.13-4.00**
All solid cancers C00-C80	1	ref	**1.65**	**1.18-2.31**	**1.77**	**1.29-2.42**	**1.79**	**1.09-2.95**
Ear, nose and throat C00-14, C31-32	1	ref	1.16	0.28-4.91	0.84	0.21-3.36	NA	NA
Esophagus C15	1	ref	0.98	0.16-5.94	0.35	0.05-2.47	NA	NA
Stomach C16	1	ref	0.78	0.05-12.57	4.19	0.54-32.50	**24.25**	**2.89-203.41**
Small intestine C17	1	ref	0.92	0.15-5.51	0.70	0.13-3.88	1.72	0.15-19.22
Colorectal C18-20	1	ref	2.31	0.85-6.28	2.10	0.81-5.41	0.66	0.08-5.63
Liver and bile ducts C22, C24	1	ref	0.29	0.03-3.25	1.42	0.29-6.95	NA	NA
Pancreas C25	1	ref	0.64	0.09-4.52	2.75	0.63-11.99	3.47	0.49-24.77
Lung C34[4]	1	ref	2.16	0.87-5.39	1.59	0.65-3.89	1.80	0.45-7.25
Skin, malignant melanoma C43	1	ref	1.06	0.41-2.75	0.56	0.21-1.48	0.63	0.08-5.10
Skin, other C44	1	ref	0.80	0.11-5.70	1.25	0.26-6.05	3.76	0.52-27.14
Soft tissue C47-49	1	ref	0.55	0.03-8.89	1.43	0.14-14.32	NA	NA
Prostate C61	1	ref	1.27	0.65-2.50	1.56	0.85-2.85	0.81	0.23-2.86
Kidney and upper urinary tract C64-C66[5]	1	ref	6.03	0.77-47.15	**7.58**	**1.01-56.94**	7.88	0.71-87.27
Bladder C67	1	ref	**5.07**	**1.16-22.09**	**5.33**	**1.27-22.43**	5.10	0.85-30.68
Brain C70-72, C75.1	1	ref	4.01	0.49-32.63	2.77	0.33-23.14	NA	NA
Thyroid C73	1	ref	0.59	0.04-9.43	0.92	0.08-10.39	NA	NA
Malignant neoplasm of other and ill-defined sites C76	1	ref	2.50	0.28-22.44	1.69	0.20-14.62	NA	NA
All haematological malignancies[6] C81-85, C88, C90-93, C95, D45, D46	1	ref	0.92	0.33-2.59	1.13	0.44-2.87	1.30	0.26-6.49
Lymphoma C81-85	1	ref	0.60	0.12-2.98	1.27	0.34-4.75	2.76	0.46-16.64
Leukaemia C91-93, C95	1	ref	1.83	0.19-17.63	1.64	0.18-14.87	NA	NA

Note: HRs reported for cancers or groups of cancers (of defined sites) with occurrence of ≥5. Please refer to the supplemental appendix Table 2 for details. Results presented only for >10 year observation time. Significant results marked with bold. C refers to diagnostic code according to the ICD-10 classification.

Abbreviations: HR, hazard ratio; SC, second cancer; CT, chemotherapy; RT, radiotherapy; CT + RT, combination of chemotherapy and radiotherapy; 95% CI, 95% confidence interval; y, years; obs, observation, NA, not available because of too few events.

[1] Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.

[2] Of the total n of 4199 with > 10 y obs time, 431 cases developed SC. Of the total n of 2974 with > 15 y obs, 340 cases developed SC. Of the total n of 1876 with > 20 y obs time, 213 cases developed SC.

[3] Analyses with >15 and >20 years done with time-dependent Cox model and a dummy variable of follow-up time (before/after 15 or 20 years).

[4] All of which were localized in the bronchi

[5] The morphology of C64 was diverse. We chose to analyze kidney and upper urinary tract together.

[6] No haematological malignancies occurred before 12 months observation time in eligible participants.

Paper II

original reports

Metachronous Contralateral Testicular Cancer the Cisplatin Era: A Population-Based Cohort Study

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abstract

PURPOSE It is hypothesized that cisplatin-based chemotherapy (CBCT) reduces the occurrence of metachronous contralateral (second) germ cell testicular cancer (TC). However, studies including treatment details are lacking. The aim of this study was to assess the second TC risk, emphasizing the impact of previous TC treatment.

PATIENTS AND METHODS Based on the Cancer Registry of Norway, 5,620 men were diagnosed with first TC between 1980 and 2009. Treatment data regarding TC were retrieved from medical records. Cumulative incidences of second TC were estimated, and standardized incidence ratios were calculated. The effect of treatment intensity was investigated using Cox proportional hazard regression.

RESULTS Median follow-up was 18.0 years, during which 218 men were diagnosed with a second TC after median 6.2 years. Overall, the 20-year crude cumulative incidence was 4.0% (95% CI, 3.5 to 4.6), with lower incidence after chemotherapy (CT) (3.2%; 95% CI, 2.5 to 4.0) than after surgery only (5.4%; 95% CI, 4.2 to 6.8). The second TC incidence was also lower for those age ≥ 30 years (2.8%; 95% CI, 2.3 to 3.4) at first TC diagnosis than those age < 30 years (6.0%; 95% CI, 5.0 to 7.1). Overall, the second TC risk was 13-fold higher compared with the risk of developing TC in the general male population (standardized incidence ratio, 13.1; 95% CI, 11.5 to 15.0). With surgery only as reference, treatment with CT significantly reduced the second TC risk (hazard ratio [HR], 0.55). For each additional CBCT cycle administered, the second TC risk decreased significantly after three, four, and more than four cycles (HRs, 0.53, 0.41, and 0.21, respectively).

CONCLUSION Age at first TC diagnosis and treatment intensity influenced the second TC risk, with significantly reduced risks after more than two CBCT cycles.

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INTRODUCTION

After being diagnosed with a primary germ cell testicular cancer (TC), the estimated 15-20–year cumulative incidence of a metachronous contralateral (second) TC is 1.9%-3.9%.[1-4] Standardized incidence ratios (SIRs), comparing the incidence of second TC with the incidence of TC in the general population, range from 12.4 to 35.7.[1-7] Treatment of the second TC will usually involve a surgical castration, leading to infertility and lifelong dependency of testosterone substitution.[8,9] From personal experience, many testicular cancer survivors (TCS) with unilateral disease fear losing their remaining testicle.

Shared etiological factors for the first and second TC, hypothesized to cause the testicular dysgenesis syndrome, represent a likely explanation for the increased incidence of a second TC.[10,11] Young age at diagnosis of the first TC is associated with the increased risk of developing a second TC.[1-4,12] The results are, however, inconclusive regarding the effect of first TC histology and subsequent second TC risk.[1,4,7,13,14]

The introduction of cisplatin in the late 1970s led to dramatically improved survival of patients with metastatic TC.[15,16] Cisplatin-based chemotherapy (CBCT) is hypothesized to reduce or delay the incidence of a metachronous contralateral TC. However, the existing literature lacks TC treatment details, if based on public registries,[1,2] involves populations screened for germ cell neoplasia in situ (GCNIS),[12,17] or includes patients treated in the precisplatin era.[3-5]

Andreassen et al[2] investigated the risk for metachronous contralateral TC in 7,102 TCS in Norway treated during 1953-2007. They found a 50% risk reduction for a second TC in men treated for metastatic compared with localized disease only for those treated after 1980, implying that this risk reduction was related to the introduction of CBCT. They emphasized that the greatest limitation of their study was the lack of TC

ASSOCIATED CONTENT

See accompanying editorial on page 265

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Does cisplatin-based chemotherapy (CBCT) reduce the risk of a metachronous contralateral (second) testicular cancer (TC)?

Knowledge Generated

The overall 20-year cumulative incidence of second TC in a population-based cohort was 4%. Treatment with CBCT significantly reduced the second TC risk, with a stronger risk reduction for each additional CBCT cycle administered. Older age at diagnosis of first TC also reduced the risk.

Relevance

Our findings add important knowledge concerning the risk of second TC. Our results are important and appreciated information for patients with TC and healthcare personnel involved in TC treatment.

treatment details. Furthermore, Fosså et al[1] conducted a large register-based study involving 29,515 TCS from the United States. They concluded that a potential dose-response relationship between cisplatin and eradication of germ cell carcinoma in situ should be investigated in future clinical studies.

The aim of this population-based study was to assess the risk of developing a metachronous contralateral TC, with emphasis on the impact of previous TC treatment including CBCT, in a national cohort with complete data on TC treatment.

PATIENTS AND METHODS

Study Cohort and Design

The Cancer Registry of Norway (CRN) identified men diagnosed with histologically verified primary germ cell TC from January 1, 1980, to December 31, 2009.[18] Major exclusion criteria included age $<$ 16 years at TC diagnosis, a prior malignancy, extragonadal germ cell cancer, and synchronous contralateral TC or death within 2 months of follow-up (Appendix Fig A1, online only). Metachronous TC was defined as a second germ cell TC diagnosed $>$ 2 months after the primary TC.

After exclusions, this historical prospective cohort study consisted of 5,620 patients with TC. Details regarding disease stage, histology, and TC treatment for first and second TCs, including relapse treatment, were retrieved from medical records. Linkage with the CRN updated through December 31, 2018, was done to ensure complete information on the incidence of second TC.

The study was approved by the Regional Committee for Medical and Health Research Ethics and the Data Protection Authorities at the University Hospital of North Norway. Passive consent from all eligible men still alive was obtained through a study information letter with the possibility to withdraw from participation, after which 23 (0.38%) men declined participation.

Staging, Treatment, and Treatment Groups

TC was staged according to the Royal Marsden Hospital staging system.[19] During the study period, the treatment principles for TC changed as previously described.[20] Adjuvant radiotherapy (RT) for stage I seminoma has gradually been abandoned, and the number of CBCT cycles applied for metastatic disease has been reduced. The use of a risk-adapted surveillance strategy or one cycle of adjuvant CBCT (nonseminoma) or carboplatin (seminoma) for stage I disease has been implemented as recommended by the Swedish and Norwegian Testicular Cancer Group (SWENOTECA).[21]

Based on total treatment burden for the first TC, the cohort was divided into four treatment groups: surgery only (including surveillance n = 1,417; 25%), chemotherapy (CT, n = 2,450; 44%), RT (n = 1,543, 27%), and both CT and RT (CT + RT, n = 210; 3.7%) (Table 1).

Statistical Methods

Continuous variables were presented with median and interquartile range (IQR), and categorical variables were presented with numbers and percent.

Follow-up was calculated from 2 months after diagnosis of the first TC until a diagnosis of a second TC, death, emigration, or December 31, 2018, whichever occurred first. Treatment was analyzed as a time-varying covariate, achieved by splitting follow-up time at exact treatment dates for each treatment modality, to avoid immortal time bias. The K-sample median test was used to test differences in median time to second TC among those developing a second TC, presented with two-sided *P*-values.

The crude cumulative incidence of metachronous contralateral TC was estimated using the Aalen-Johansen estimator,[22] with death of any cause as a competing risk. To compare the incidence of metachronous contralateral TC to the incidence of TC in the general population, SIRs were calculated. The estimates were obtained by dividing the number of metachronous contralateral TCs in the cohort to the expected number of metachronous contralateral

TABLE 1. Patient Characteristics at First Primary TC Diagnosis

Characteristic	Total at Risk (N = 5,620)	Individuals Without Second TC (n = 5,402)	Individuals Developing Second TC (n = 218)
Decade of first TC diagnosis			
1980-1989	1,287 (23)	1,228 (23)	59 (27)
1990-1999	1,897 (34)	1,824 (34)	73 (34)
2000-2009	2,436 (43)	2,350 (43)	86 (39)
Follow-up, years, median (IQR)[a]	18.0 (12.0-25.5)	18.5 (12.5-25.8)	6.2 (3.3-10.6)[b]
Age at diagnosis, years, median (IQR)	33.0 (27.2-40.9)	33.3 (27.3-41.2)	28.7 (24.6-33.5)
Age at diagnosis, dichotomized, years			
< 30	2,124 (38)	1,999 (37)	125 (57)
≥ 30	3,496 (62)	3,403 (63)	93 (43)
Histology			
Seminoma	2,938 (52)	2,831 (52)	107 (49)
Nonseminoma	2,682 (48)	2,571 (48)	111 (51)
Initial disease stage[c]			
I	3,942 (70)	3,766 (70)	176 (80)
Mk+/II	1,127 (20)	1,097 (20)	30 (14)
III	116 (2.1)	114 (2.1)	2 (1.0)
IV	435 (7.7)	425 (7.9)	10 (4.6)
Treatment[d]			
Surgery only[e]	1,417 (25)	1,345[f] (25)	72 (33)
CT	2,450 (44)	2,379 (44)	71 (32)
RT	1,543 (27)	1,471 (27)	72 (33)
CT + RT	210 (3.7)	207 (3.8)	3 (1.4)
Cause of first-line CT			
Adjuvant, CS I	843 (32)	811 (31)	32 (43)
Primary metastatic disease	1,538 (58)	1,502 (58)	36 (49)
Recurrence	279 (10)	273 (11)	6 (8.1)
First CT regimen			
BEP-20	1,507 (57)	1,464 (57)	43 (58)
CVB	367 (14)	357 (14)	10 (13.5)
EP	241 (9.1)	237 (9.2)	4 (5.4)
Other CBCT[g]	184 (6.9)	180 (6.9)	4 (5.4)
Adjuvant carboplatin[h]	295 (11)	285 (11)	10 (13.5)
CEB	44 (1.6)	42 (1.6)	2 (2.7)
Other[i]	22 (0.8)	21 (0.8)	1 (1.4)
No. of CBCT cycles[j]			
1	220 (9.5)	210 (9.3)	10 (16)
2	319 (14)	307 (14)	12 (20)
3	439 (19)	427 (19)	12 (20)
4	1,028 (44)	1,004 (44)	24 (39)
> 4	320 (14)	317 (14)	3 (4.9)
RT first field			
L-Field[k]	1,388 (79)	1,321 (79)	67 (89)
Para-aortal	267 (15)	260 (15)	7 (9.3)

(continued on following page)

TABLE 1. Patient Characteristics at First Primary TC Diagnosis (continued)

Characteristic	Total at Risk (N = 5,620)	Individuals Without Second TC (n = 5,402)	Individuals Developing Second TC (n = 218)
Supradiaphragmatic	13 (0.7)	12 (0.7)	1 (1.3)
Supra- and infradiaphragmatic[l]	21 (1.2)	21 (1.3)	0
Other[m]	64 (3.6)	64 (3.8)	0
RT dose for first RT field, Gy			
1-20	13 (0.7)	12 (0.7)	1 (1.3)
20-29	514 (29)	490 (29)	24 (32)
30-39	986 (56)	943 (56)	43 (57)
≥ 40	240 (14)	233 (14)	7 (9.3)

NOTE. Data are presented as n (%), unless otherwise stated.

Abbreviations: BEP-20, bleomycin, etoposide, and cisplatin; CBCT, cisplatin-based chemotherapy; CEB, carboplatin, etoposide, and bleomycin; CS I, clinical stage I; CT, chemotherapy; CT + RT, combination of CT and RT; CVB, cisplatin, vinblastine, and bleomycin; EP, etoposide and cisplatin; Gy, gray; IQR, interquartile range; Mk+, marker positive; RT, radiotherapy; TC, testicular cancer.

[a]Follow-up until diagnosis of metachronous contralateral TC, death, emigration, or December 31, 2018, whichever occurred first.

[b]The longest time interval between first TC and second TC was 27.1 years.

[c]As described by Peckham et al.[19]

[d]Based on total treatment burden.

[e]The surgery-only group included men followed with surveillance after orchiectomy (n = 1,167; 21%) and men who underwent additional retroperitoneal lymph node dissection without CT or RT (n = 250; 4.4%).

[f]Two men included in the surgery-only group were diagnosed with clinical stage IV. One refused treatment, and the other was no candidate for treatment. They both died shortly (but > 2 months) after diagnosis.

[g]Of which a total of 141 were dose-escalated CBCT.

[h]Fifteen of the 295 men initially treated with adjuvant carboplatin were subsequently treated with CBCT and, as a consequence, analyzed according to the total number of CBCT cycles. Also, one person had RT in addition to carboplatin. Of the 279 men treated with adjuvant carboplatin monotherapy included in the Cox regression analysis, 273 received one cycle and 6 received two cycles.

[i]Carboplatin monotherapy in metastatic setting (n = 17), cyclophosphamide/adriamycin (n = 1), CAOS (actinomycin D, adriamycin, vincristine, cyclophosphamide) (n = 3), actinomycin D (n = 1).

[j]May have received additional CT regimes, but these are not accounted for in this number. A total of 334 men received non-CBCT, which are not included here.

[k]L-Field or dogleg-field. Also included in this category are 53 individuals who received RT of groin in addition to L-field and two individuals who received a reversed Y-field.

[l]Sixteen of 21 individuals received infradiaphragmatic RT as first RT-field and a short while later received supradiaphragmatic RT.

[m]RT toward bone (n = 21), CNS (n = 21), abdominal residual masses (n = 16), intraoperative RT (n = 1), skin lesions (n = 1), nonspecified sites (n = 4).

TC, given the incidence of TC in a comparable Norwegian male population, matched by 5-year age groups and calendar year of follow-up. Cumulative incidences and SIRs with respective 95% CIs were calculated for the whole cohort and stratified according to treatment groups, age at diagnosis, follow-up time, and histology.

The effect of treatment and histology on the second TC risk were evaluated using Cox proportional hazard regression models with time since diagnosis as time scale, the surgery-only group as reference, and adjusting for age at diagnosis.[20] Additionally, histology as a risk factor was investigated in a multivariable Cox regression model which included treatment. Cumulative CT doses were estimated based on the CT regimen and number of CT cycles. The Cox regression model was also used to evaluate the effect of age at diagnosis (dichotomized). A nonsignificant Schoenfeld test showed that the proportional hazard assumption was met for all analyses, except for cumulative doses (Appendix Table A1, online only) and the dichotomized age variable ($P = .049$). For the latter, the proportional hazard assumption was judged to be met by visual inspection of a log-log survival plot. The results are presented as hazard ratios (HRs) with corresponding 95% CIs and *P*-values.

Data were analyzed using Stata statistical software (version MP 16.1; STATA, College Station, TX). A *P*-value $< .05$ was considered significant.

RESULTS

Characteristics of the Total Study Cohort and the Metachronous TC Subcohort

The total study cohort consisted of 5,620 men with a median follow-up time of 18 years (IQR, 12.0-25.5) (Table 1). Median age at diagnosis was 33 years, 38% were < 30 years, and 70% were diagnosed with stage I disease at first TC. Overall, 25% were treated with surgery only, and 44% were treated with CT at first TC.

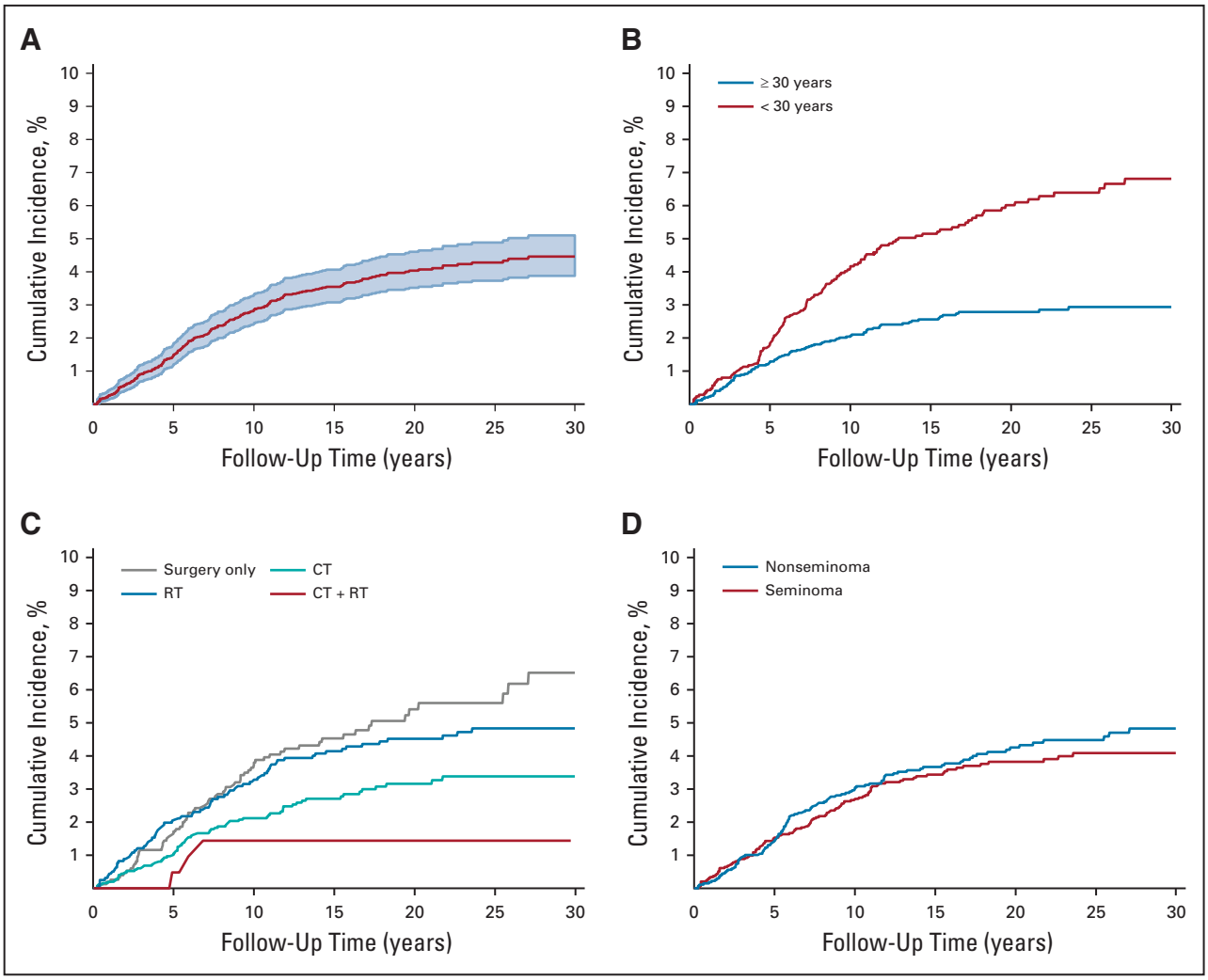


FIG 1. Crude cumulative incidences of metachronous contralateral TC by follow-up time. (A) All patients (with 95% CI), (B) by age at first TC, dichotomized (C) by treatment groups at first TC, and (D) by histology at first TC. In (A), the red line indicates the incidence of metachronous contralateral TC, and the blue area indicates the 95% CI. CT, chemotherapy; CT + RT, combination of CT and RT; RT, radiotherapy; TC, testicular cancer.

Overall, 218 (3.9%) men developed a metachronous contralateral TC after median 6.2 years (IQR, 3.3-10.6) (Table 1). Among these 218 men, median age at first TC diagnosis was 28.7 years, 57% were < 30 years at diagnosis of first TC, and seminoma (49%) and nonseminoma (51%) histology of the first TC was equally distributed. Furthermore, 80% were diagnosed with clinical stage I at first TC, and as treatment for first TC, 33% had surgery only and 32% received CT. Median time to second TC did not differ according to treatment ($P = .55$) or age at diagnosis of first TC ($P = .10$) (Appendix Table A2, online only).

The majority of the second TCs were seminomas (72%) (Appendix Table A3, online only). At diagnosis of the second TC, 84% had stage I disease and 53% were treated with surgery only.

Cumulative Incidences of Second TC

The overall crude cumulative second TC incidence was 4.0% (95% CI, 3.5 to 4.6) at 20 years (Fig 1A, Table 2). The second TC incidence was lower in those age ≥ 30 years at first TC diagnosis (2.8%; 95% CI, 2.3 to 3.4) than in those age < 30 years (6.0%; 95% CI, 5.0 to 7.1) (Fig 1B). The second TC incidence was also lower after treatment with CT (3.2%; 95% CI, 2.5 to 4.0) and CT + RT at first TC (1.4%; 95% CI, 0.4 to 3.9) than after surgery only (5.4%; 95% CI, 4.2 to 6.8) or RT (4.5%; 95% CI, 3.6 to 5.6) (Fig 1C).

For those age < 30 years at first TC diagnosis, 20-year cumulative incidence after surgery only was 8.0% (95% CI, 5.8 to 10.6), and after CT, it was 4.8% (95% CI, 3.6 to 6.3) (Table 2). In comparison, for those age ≥ 30 years at first TC diagnosis, the second TC incidence was 3.2% (95% CI,

TABLE 2. Cumulative Incidences of Metachronous Contralateral TC According to Treatment, Age, and Histology at First TC and Specified Follow-up Time

	< 5 years			< 10 years			< 15 years			< 20 years		
Variable	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Total	82	1.5	1.2 to 1.8	157	2.8	2.4 to 3.3	192	3.5	3.1 to 4.1	209	4.0	3.5 to 4.6
Age at diagnosis, years												
< 30	38	1.8	1.3 to 2.4	86	4.1	3.3 to 5.0	106	5.2	4.3 to 6.2	118	6.0	5.0 to 7.1
≥ 30	44	1.3	0.9 to 1.7	71	2.0	1.6 to 2.6	86	2.6	2.1 to 3.1	91	2.8	2.3 to 3.4
Treatment, all patients												
Surgery only[a]	24	1.6	1.1 to 2.4	52	3.6	2.8 to 4.7	62	4.5	3.5 to 5.7	68	5.4	4.2 to 6.8
CT	25	1.0	0.7 to 1.5	52	2.1	1.6 to 2.7	63	2.7	2.1 to 3.4	69	3.2	2.5 to 4.0
RT	32	2.0	1.4 to 2.8	50	3.2	2.4 to 4.2	64	4.1	3.2 to 5.2	69	4.5	3.6 to 5.6
CT + RT	1	0.5	0.1 to 2.5	3	1.4	0.4 to 3.9	3	1.4	0.4 to 3.9	3	1.4	0.4 to 3.9
Treatment, age < 30 years												
Surgery only[a]	11	1.8	1.0 to 3.1	28	4.7	3.2 to 6.6	36	6.3	4.5 to 8.5	42	8.0	5.8 to 10.6
CT	18	1.6	1.0 to 2.5	40	3.6	2.6 to 4.8	46	4.2	3.1 to 5.6	50	4.8	3.6 to 6.3
RT	9	2.5	1.2 to 4.6	16	4.5	2.7 to 7.0	22	6.3	4.0 to 9.1	24	6.9	4.6 to 10.0
CT + RT	0	0	0	2	2.9	0.6 to 9.1	2	2.9	0.6 to 9.1	2	2.9	0.6 to 9.1
Treatment, age ≥ 30 years												
Surgery only[a]	13	1.5	0.9 to 2.5	24	2.9	1.9 to 4.2	26	3.2	2.1 to 4.6	26	3.2	2.1 to 4.6
CT	7	0.5	0.2 to 1.0	12	0.9	0.5 to 1.5	17	1.4	0.9 to 2.2	19	1.7	1.1 to 2.7
RT	23	1.9	1.2 to 2.8	34	2.8	2.0 to 3.9	42	3.5	2.6 to 4.7	45	3.8	2.8 to 5.0
CT + RT	1	0.7	0.1 to 3.6	1	0.7	0.1 to 3.6	1	0.7	0.1 to 3.6	1	0.7	0.1 to 3.6
Histology, all patients												
Seminoma	44	1.5	1.1 to 2.0	78	2.7	2.1 to 3.3	97	3.4	2.8 to 4.2	104	3.8	3.1 to 4.6
Nonseminoma	38	1.4	1.0 to 1.9	79	3.0	2.4 to 3.7	95	3.7	3.0 to 4.4	105	4.3	3.5 to 5.1
Histology, age < 30 years												
Seminoma	13	2.1	1.2 to 3.4	26	4.2	2.8 to 5.9	35	5.8	4.1 to 7.9	38	6.6	4.7 to 8.8
Nonseminoma	25	1.7	1.1 to 2.4	60	4.0	3.1 to 5.1	71	4.9	3.8 to 6.1	80	5.8	4.6 to 7.1
Histology, age ≥ 30 years												
Seminoma	31	1.3	0.9 to 1.9	52	2.3	1.7 to 2.9	62	2.8	2.2 to 3.5	66	3.1	2.4 to 3.9
Nonseminoma	13	1.1	0.6 to 1.8	19	1.6	1.0 to 2.5	24	2.1	1.4 to 3.1	25	2.3	1.5 to 3.3

NOTE. n refers to the cumulative number of men developing metachronous contralateral TC up until specified follow-up time. Age refers to age at diagnosis of first TC, dichotomized on < 30 or ≥ 30 years.

Abbreviations: CT, chemotherapy; CT + RT, combination of CT and RT; RT, radiotherapy; TC, testicular cancer.

[a]Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.

2.1 to 4.6) after surgery only and 1.7% (95% CI, 1.1 to 2.7) after CT.

The second TC incidence did not differ according to first TC histology, with estimates of 3.8% (95% CI, 3.1 to 4.6) after seminoma and 4.3% (95% CI, 3.5 to 5.1) after nonseminoma (Fig 1D).

Risk of Second TC in Relation to the General Population

Overall, the second TC risk was 13-fold higher compared with the risk of developing TC in the general population (SIR, 13.1; 95% CI, 11.5 to 15.0) (Table 3). The risk was lower after treatment with CT (SIR, 9.1; 95% CI, 7.2 to 11.5) and CT + RT (SIR, 8.6; 95% CI, 2.8 to 26.7) at first TC than after surgery only (SIR, 16.3; 95% CI, 12.9 to 20.5) and RT (SIR, 17.7; 95% CI, 14.1 to 22.3). SIRs decreased with increasing age at diagnosis and was highest for those age 20-30 years (SIR, 14.0; 95% CI, 11.7 to 16.8.). The risk for a second TC was the highest within the first 5 years of follow-up after diagnosis of the first TC (SIR, 17.0; 95% CI, 13.7 to 21.2) and decreased with increasing follow-up time.

HRs for Second TC

With surgery only as the reference group, the second TC risk was significantly lower after treatment with CT at first TC (HR, 0.55; 95% CI, 0.40 to 0.76) (Table 4). A sensitivity analysis excluding those treated with CT other than CBCT

TABLE 3. SIRs for Metachronous Contralateral TC According to Treatment, Age, and Histology at First TC and Follow-up Time

Variable	No. of Events	SIR	95% CI
Total	218	13.1	11.5 to 15.0
Treatment, first TC			
Surgery only[a]	72	16.3	12.9 to 20.5
CT	71	9.1	7.2 to 11.5
RT	72	17.7	14.1 to 22.3
CT + RT	3	8.6	2.8 to 26.7
Age, dichotomized, years			
< 30	125	13.4	11.2 to 15.9
≥ 30	93	12.8	10.4 to 15.7
Age at diagnosis, years			
16-20	9	8.5	4.4 to 16.4
20-30	116	14.0	11.7 to 16.8
30-40	76	13.6	10.9 to 17.0
40-50	14	10.3	6.1 to 17.4
> 50	3	9.6	3.1 to 29.6
Histology			
Seminoma	107	14.7	12.2 to 17.8
Nonseminoma	111	11.9	9.9 to 14.3
Follow-up time, years			
< 5	82	17.0	13.7 to 21.2
5-10	75	15.5	12.3 to 19.4
10-15	35	10.4	7.4 to 14.4
15-20	17	8.7	5.4 to 13.9
> 20[b]	9	5.6	2.9 to 10.7

Abbreviations: CT, chemotherapy; CT + RT, combination of CT and RT; RT, radiotherapy; SIR, standardized incidence ratio; TC, testicular cancer.

[a]Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.

[b]The longest time interval between first TC and second TC was 27.1 years.

(carboplatin-based, n = 332; other CT, n = 2) was performed with no significant change of results (data not shown). Treatment with RT did not affect the second TC risk (HR, 1.10; 95% CI, 0.79 to 1.54).

For each additional CBCT cycle administered, the point estimates for second TC risk decreased, with significantly reduced risks after three (HR, 0.53; 95% CI, 0.29 to 0.97), four (HR, 0.41; 95% CI, 0.25 to 0.66), and more than four cycles (HR, 0.21; 95% CI, 0.07 to 0.66) (Table 4, Fig 2). The hazard of second TC was not significantly different after treatment with adjuvant carboplatin monotherapy (HR, 1.22; 95% CI, 0.62 to 2.39). For each increase of 100 mg/m^2 cisplatin, the second TC risk decreased equivalent to the results according to the number of CBCT cycles. The effect on second TC risk was weakened for the dose level of 101-200 mg/m^2 when carboplatin was included in the analysis of cumulative platinum doses (Appendix Table A1).

The second TC risk was significantly reduced for those age ≥ 30 years at first TC diagnosis (HR, 0.47; 95% CI, 0.36 to 0.62). In age-adjusted Cox regression, nonseminoma histology at first TC was associated with a decreased risk of second TC (HR, 0.73; 95% CI, 0.55 to 0.98). However, compared with seminoma, this association disappeared when treatment at first TC was included in the model (HR, 0.97; 95% CI, 0.65 to 1.45) (Table 4).

DISCUSSION

In this population-based study, the overall 20-year cumulative incidence of a metachronous TC was 4.0% in a well-described cohort with complete information on total treatment burden and a long follow-up time. We demonstrated, to the best of our knowledge for the first time, that the risk of a metachronous contralateral TC decreased with each additional CBCT cycle administered, with significantly reduced risks after more than two CBCT cycles.

The overall second TC cumulative incidence of 4% and total SIR of 13.1 found in this study are in accordance with the existing literature.[1-7] We found a reduced second TC risk after treatment with CT at first TC, and our results lend strong support to the hypothesis that cisplatin reduces the second TC risk.[1-3,5] Treatment with RT has not been considered to affect the TC incidence,[3,4,12] and our results are in agreement with this. Adjuvant infradiaphragmatic RT after seminoma results in a total dose of 0.09-0.32 Gy of scattered radiation to the remaining testicle, which is probably insufficient for eradication of GCNIS if present.[23]

GCNIS is the precursor of germ cell TC.[24] If left untreated for 5 years, 50% of patients with GCNIS will develop an invasive cancer.[25] There has not been a tradition to screen for GCNIS in Norway during the study period as it has only been performed in selected high-risk patients.[17,26,27] Metastatic TC is highly sensitive to cisplatin. However, cisplatin seems to have a modest but possibly dose-dependent effect on eradication of GCNIS.[17,25,28-31] In the present study, we found a strong association between the number of CBCT cycles, cumulative cisplatin dose, and the second TC risk. Our results are in line with the study by Brabrand et al,[17] who found significantly reduced second TC risk after more than four compared with one to three CBCT cycles or no CT in a study of 61 TCS with biopsy-proven GCNIS in the contralateral testicle. We found no risk reduction after one to two CBCT cycles, which corroborates results from a prospective study on second TC risk after one to two adjuvant CBCT cycles in patients with stage I nonseminoma.[26] In contrast to the results from the randomized trial by Oliver et al[32] comparing adjuvant carboplatin with RT, we found no decrease of second TC risk after treatment with adjuvant carboplatin.

TABLE 4. Age-Adjusted HRs for Metachronous Contralateral TC According to Treatment Groups, Treatment Intensity, Age, and Histology at First TC

Variable	HR	95% CI	P
Treatment			
Surgery only	1	Ref	Ref
CT	**0.55**	**0.40 to 0.76**	**< .001**
RT	1.10	0.79 to 1.54	.580
CT + RT	0.50	0.16 to 1.57	.233
No. of CBCT cycles			
Surgery only	1	Ref	Ref
1	1.01	0.52 to 1.96	.983
2	0.74	0.40 to 1.36	.332
3	**0.53**	**0.29 to 0.97**	**.040**
4	**0.41**	**0.25 to 0.66**	**< .001**
> 4	**0.21**	**0.07 to 0.66**	**.008**
Carboplatin, adjuvant[a]	1.22	0.62 to 2.39	.565
RT field			
Surgery only	1	Ref	Ref
L-Field	1.17	0.78 to 1.62	.521
Para-aortal	0.75	0.34 to 1.64	.468
RT dose for first abdominal RT-field, Gy			
Surgery only	1	Ref	Ref
20-29	1.24	0.77 to 1.98	.383
30-39	1.04	0.70 to 1.55	.832
≥ 40	1.17	0.50 to 2.70	.721
Age at diagnosis,[b] years			
< 30	1	Ref	Ref
≥ 30	**0.47**	**0.36 to 0.62**	**< .001**
Histology			
Age-adjusted			
Seminoma	1	Ref	Ref
Nonseminoma	**0.73**	**0.55 to 0.98**	**.034**
Multivariable[c]			
Seminoma	1	Ref	Ref
Nonseminoma	0.97	0.65 to 1.45	.883

NOTE. Significant results marked with bold. Age refers to age at diagnosis of first TC, dichotomized on < 30 or ≥ 30 years.

Abbreviations: CBCT, cisplatin-based chemotherapy; CT, chemotherapy; CT + RT, combination of CT and RT; Gy, gray; HR, hazard ratio; Ref, reference; RT, radiotherapy; TC, testicular cancer.

[a]Carboplatin monotherapy, carboplatin in adjuvant setting for stage I seminoma.

[b]Not age adjusted.

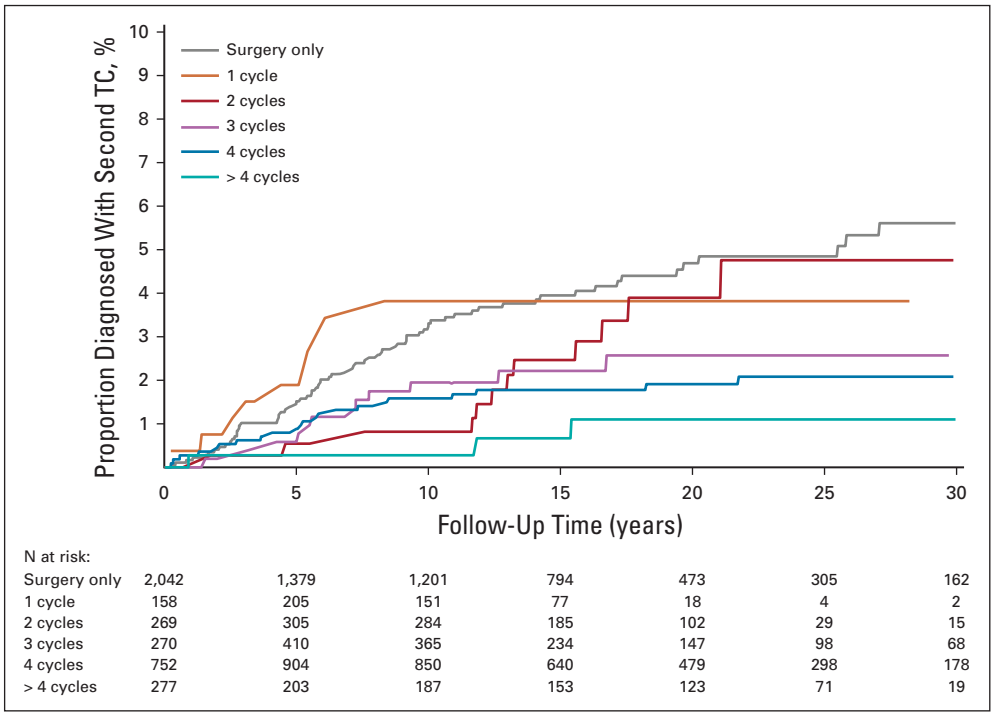
[c]Adjusted for treatment in addition to age.

The modulating effect of the blood-testis barrier on the intratubular concentration of cytotoxic drugs,[33,34] possibly in part, explains the need for higher cumulative doses of cisplatin before effect on GCNIS and the subsequent second TC risk. However, cisplatin undoubtedly has an effect on the testis, demonstrated by the decrease of sperm concentration and quality and the changes of sperm DNA following CBCT.[35-37] Furthermore, there seems to be a relationship between the number of CBCT cycles and the recovery of spermatogenesis.[36-39] A recent publication by Weibring et al[40] did not find a long-term reduction of sperm count after one cycle of CBCT. On the other hand, three or more cycles of CBCT may lead to long-term or permanent impairment of sperm function.[36-38]

It has been suggested that cisplatin delays rather than reduces the development of a second TC.[29,31] In accordance with Schaapveld et al,[3] our results do not lend support to this hypothesis. On the contrary, we found that there was a longer median time interval between first TC and second TC after surgery only (7.0 years) than after CT (5.8 years), although not statistically significant. The overall latency of 6.2 years between first TC and second TC agrees with previous studies.[1-3] In the present study, with a very long follow-up time of median 18 years, 72% of second TCs developed within 10 years of follow-up. This is in line with the report of a plateau in incidence after 15-20 years.[3,4] However, second TCs may occur late,[41] and the longest time interval between first TC and second TC in our cohort was 27 years.

A polygenic susceptibility, coupled with fetal and early-life environmental factors, is involved in TC development.[10,11,42-45] The shared prenatal predisposition of the first and second TC probably accounts for the increased risk of metachronous contralateral TC, and the increased risk in younger versus older men is in turn presumably explained by this.[1,46] Young age at TC diagnosis has been established as an important risk factor for developing metachronous contralateral TC,[1-4,12,13,47] and our results are in complete agreement with this. In our study, median age at diagnosis of first TC was 4.6 years younger in men who later developed a second TC than men with unilateral TC. Furthermore, men age < 30 years at first TC had more than twice as high 20-year cumulative second TC incidence than those 30 years or older at first TC diagnosis.

The current knowledge regarding histology and the risk of metachronous contralateral TC is inconsistent.[1,4,7,13,14] Studies conducted in the precisplatin era found a higher risk for metachronous contralateral TC after nonseminoma than after seminoma.[4,14] In the cisplatin era, some studies concluded with the opposite,[7,13] supporting an effect of CBCT.[1] We found no association between first TC histology and the risk of a second TC when adjusting for age and treatment, which is in line with Andreassen et al.[2] Our results suggest that the differences found in histology[1,4,7,13,14] are in fact caused by the effect of CBCT, as patients with nonseminoma



N at risk:							
Surgery only	2,042	1,379	1,201	794	473	305	162
1 cycle	158	205	151	77	18	4	2
2 cycles	269	305	284	185	102	29	15
3 cycles	270	410	365	234	147	98	68
4 cycles	752	904	850	640	479	298	178
> 4 cycles	277	203	187	153	123	71	19

FIG 2. Proportion diagnosed with metachronous contralateral TC by follow-up time and the number of cisplatin-based chemotherapy cycles, adjusted for age at TC diagnosis. The risk table presents the crude number of individuals by follow-up time. TC, testicular cancer.

are more often treated with CBCT than patients with seminoma.

In a recent review by Zequi et al,[48] 60.4% of metachronous contralateral TCs had a seminoma histology. This is in line with the present study in which 72% of the second TCs were seminomas. The abundancy of seminoma histology of second TCs is probably caused by age.[18]

In our study, the majority (84%) of second TCs were diagnosed as clinical stage I, and this correlates with the results published in the review by Zequi et al[48] (73.3% in stage I). Our even higher proportion diagnosed in stage I might be a result of robust follow-up procedures, centralized treatment of TC in Norway, and the risk-adapted biopsy strategy of the contralateral testicle.[27,49]

Important strengths of our study include the consideration of a nationwide cohort, the completeness of cancer incidence rates of the CRN,[18] and the complete information on treatment burden in a large and unselected study cohort with a long follow-up time. The risk-adapted treatment strategy in clinical stage I disease recommended by SWENOTECA has made it possible to compare adjuvant CT with the surveillance strategy.[21]

The lack of information regarding GCNIS and risk factors for TC, such as family history of TC, history of cryptorchidism, or infertility, are potential limitations. Tissue samples available for genetic analyses could have been of particular interest.

In conclusion, we found a strong association between the number of CBCT cycles and the subsequent risk of a metachronous contralateral TC. Patients with metastatic unilateral TC might appreciate information on the significant risk reduction of second TC after treatment with CT. Although most second TCs develop within 10 years after diagnosis of the first TC, they may develop after more than 20 years. It is important that TCS are aware of this risk and that the importance of regular lifelong self-examination is emphasized.

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DISCLAIMER

The study has used data from the Cancer Registry of Norway. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway is intended nor should be inferred.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Metachronous Contralateral Testicular Cancer in the Cisplatin Era: A Population-Based Cohort Study

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No potential conflicts of interest were reported.

APPENDIX

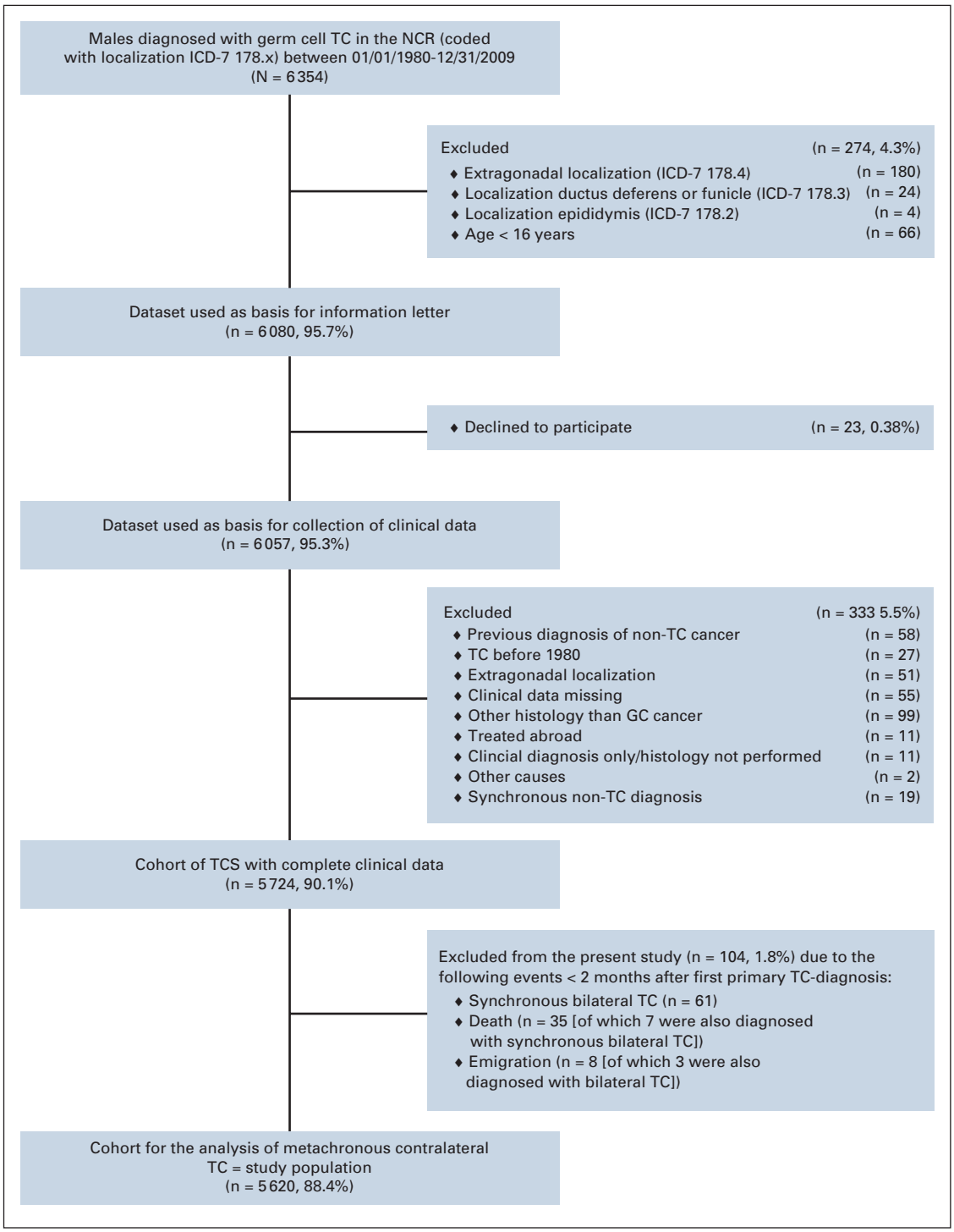

FIG A1. Flowchart presenting the study cohort. GC, germ cell; ICD-7, International Classification of Diseases Version 7; NCR, the Norwegian Cancer Registry; TC, testicular cancer; TCS, testicular cancer survivors.

TABLE A1. Age-Adjusted HRs for Metachronous Contralateral TC According to Cumulative Cisplatin, Platinum, and Bleomycin Doses at First TC[a]

Chemotherapy Dose	HR	95% CI	*P*
Cumulative cisplatin dose, mg/m^2			
Surgery only	1	Reference	Reference
1-100	1.01	0.52 to 1.96	.984
101-200	0.74	0.40 to 1.36	.331
201-300	**0.53**	**0.29 to 0.98**	**.043**
301-400	**0.43**	**0.27 to 0.70**	**.001**
> 400	**0.14**	**0.03 to 0.52**	**.004**
Carboplatin	1.15	0.62 to 2.12	.667
Cumulative total platinum dose, mg/m^{2b}			
Surgery only	1	Reference	Reference
1-100	1.01	0.52 to 1.96	.984
101-200	0.91	0.56 to 1.47	.697
201-300	**0.53**	**0.29 to 0.99**	**.045**
301-400	**0.46**	**0.29 to 0.72**	**.001**
> 400	**0.12**	**0.03 to 0.50**	**.003**
Cumulative bleomycin dose, IU			
Surgery only	1	Reference	Reference
1-100,000	0.92	0.50 to 1.70	.789
100,001-200,000	0.55	0.26 to 1.14	.107
200,001-300,000	**0.46**	**0.30 to 0.69**	**<.001**
> 300,000	0.29	0.07 to 1.19	.086
Chemotherapy without bleomycin	0.84	0.47 to 1.50	.550

NOTE. Significant results marked with bold.

Abbreviations: HR, hazard ratio; TC, testicular cancer.

[a]When analyzing the effect of cumulative doses, the proportional hazard assumption was violated for some treatment groups. We fitted new models with an interaction effect between follow-up time and the selected treatment groups and compared model fit using BIC. In all cases, the best fit was provided by the simple model without interaction effects, and hence, the results from these are presented.

[b]Cumulative total platinum doses contain cumulative doses of cisplatin and/or carboplatin. For carboplatin, the corresponding cisplatin-equivalent doses were estimated by dividing the carboplatin doses by four (Ozols Cancer Treat Rev. 1985).

TABLE A2. Time to Metachronous Contralateral TC According to Characteristics at First TC

Characteristic	Individuals Developing Metachronous Contralateral TC (n = 218)
By time since treatment at first TC, years	
Surgery only[a]	7.0 (4.3-10.0)
CT	5.8 (3.2-10.9)
RT	6.5 (2.7-10.7)
CT + RT	5.9 (4.9-6.2)
By age at first TC, dichotomized, years	
< 30 years	7.2 (4.4-10.9)
≥ 30 years	5.3 (2.6-9.3)
By histology at first TC, years	
Seminoma	6.7 (3.2-10.5)
Nonseminoma	5.9 (4.1-10.9)

NOTE. Data are presented as median (IQR).
Abbreviations: CT, chemotherapy; CT + RT, combination of CT and RT; IQR, interquartile range; RT, radiotherapy; TC, testicular cancer.
[a]Includes men treated with surveillance and men treated with retroperitoneal lymph node dissection in addition to orchiectomy.

TABLE A3. Patient Characteristics at Diagnosis of Metachronous Contralateral TC

Characteristic	Individuals Developing Metachronous Contralateral TC (n = 218)
Histology, second TC	
Seminoma	157 (72)
Nonseminoma	58 (27)
Missing	3 (1.4)
Disease stage, second TC[a]	
I	184 (84)
Mk+ and II	16 (7.3)
III	3 (1.4)
IV	4 (1.8)
Missing	11 (5.1)
Treatment, second TC	
Surgery only	115 (53)
CT[b]	71 (33)
RT	16 (7.3)
CT + RT	0
Missing	16 (7.3)

NOTE. Data are presented as n (%).
Abbreviations: CT, chemotherapy; CT + RT, combination of CT and RT; Mk+, marker positive; RT, radiotherapy; TC, testicular cancer.
[a]As described by Peckham et al.[19]
[b]Of which one had disseminated synchronous nongerm cell SC (C34) treated with CT. A few of these cases were originally treated with surveillance but received CT at relapse.

Paper III

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Testicular Cancer in the Cisplatin Era: Causes of Death and Mortality Rates in a Population-Based Cohort

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Running head: Mortality after testicular cancer treated in the cisplatin era

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Abstract

Purpose

Using complete information regarding testicular cancer (TC) treatment burden, this study aimed to investigate cause-specific non-TC mortality with impact on previous treatment with platinum-based chemotherapy (PBCT) or radiotherapy (RT).

Patients and methods

Overall, 5707 men, identified by the Cancer Registry of Norway diagnosed with TC 1980-2009, were included in this population-based cohort study. By linking data with the Norwegian Cause of Death Registry, standardized mortality ratios (SMRs), absolute excess risks (AERs) and adjusted hazard ratios (HRs) were calculated.

Results

Median follow-up was 18.7 years, during which non-TC death was registered for 665 (12%) men. The overall excess non-TC mortality was 23% (SMR 1.23, 95% CI 1.14-1.33, AER 11.14) compared with the general population, with increased risks after PBCT (SMR 1.23, 95% CI 1.07-1.43, AER 7.68) and RT (SMR 1.28, 1.15-1.43, AER 19.55), but not after surgery. The highest non-TC mortality was observed in those <20 years at TC diagnosis (SMR 2.27, 95% CI 1.32-3.90, AER 14.42). The most important cause of death was non-TC second cancer with an overall SMR of 1.53 (95% CI 1.35-1.73, AER 7.94), with significantly increased risks after PBCT and RT. Overall non-cancer mortality was increased by 15% (SMR 1.15, 95% CI 1.04-1.27, AER 4.71). Importantly, excess suicides appeared after PBCT (SMR 1.65, 95% CI 1.01-2.69, AER 1.39). Compared with surgery, increased non-TC mortality appeared after 3 (HR 1.47, 95% CI 0.91-2.39) 4 (HR 1.41, 95% CI 1.01-1.99) and >4 (HR 2.04, 95% CI 1.25-3.35) cisplatin-based chemotherapy cycles after >10 years of follow-up.

Conclusion

TC treatment with PBCT or RT is associated with a significant excess risk of non-TC mortality, and increased risks emerged after >2 cisplatin-based chemotherapy cycles after >10 years follow-up.

Introduction

The excellent cure rates of germ cell testicular cancer (TC), predominantly affecting men aged 20-40 years, has led to a growing number of long-term TC survivors (TCS).[1] However, their future is threatened by increased mortality rates compared with the general population 20-30 years after the TC diagnosis.[2] The inferior survival is presumed to be related to previous cytotoxic treatment.[3]

Platinum-based chemotherapy (PBCT) was introduced in the late 1970s and has been crucial for the exceptional improvement in survival.[4] On the downside, this life-saving treatment has been linked with life-threatening late effects such as cardiovascular disease (CVD)[5-7] and second cancers (SC).[8-11] Radiotherapy (RT) was until the early 2000s the standard treatment of localized and small-volume metastatic seminoma.[12] RT has been abandoned as standard treatment of seminomas in many countries, in part because of the strong association between treatment with RT and SC risk.[8,9,11,13,14]

Previous PBCT[6,9,15,16] and RT[9,16-19] is related to increased non-TC mortality compared with the general population, and in particular deaths due to non-TC SCs.[9,16] Excess non-cancer mortality has also been described,[15] with an association between PBCT and increased cardiovascular mortality.[5,6,16] However, analyses regarding detailed treatment data and its impact on overall and cause-specific mortality are sparsely described.

The aim of the present study was to assess non-TC mortality and causes of death in relation to TC treatment, including the impact of number of cisplatin-based chemotherapy cycles, in a population-based cohort with complete information on TC treatment burden.

Methods

Study cohort and design

Through the Cancer Registry of Norway (CRN), men diagnosed with histologically verified germ cell TC from January 1, 1980 to December 31, 2009 were identified.[20] Principal exclusion criteria included a prior malignancy, age <16 years at TC diagnosis, extragonadal germ cell cancer or missing clinical data (Supplemental Figure S1).

The final study population in this historical prospective cohort study comprised 5707 TC patients. Detailed information regarding disease stage, histology and complete TC treatment, including relapse treatment, was collected from medical records. The clinical database was linked with data from the Norwegian Cause of Death Registry (NCoDR) updated through December 31, 2018. Mortality due to TC was not the scope of this study. Accordingly, SC refers to non-TC SC.

The Regional Committee for Medical and Health Research Ethics has approved the study (2014/1745). Passive consent was obtained through a study information letter distributed to all eligible men still alive, upon which 24 (0.39%) declined participation.

Staging, Treatment and Treatment Groups

The staging of TC was performed according to the Royal Marsden Hospital staging system.[21] The TC treatment principles were modified during the study period.[8] The number of PBCT cycles used to treat metastatic TC has been reduced, adjuvant RT for stage I seminoma was gradually discontinued, and a risk-adapted strategy with surveillance or 1 cycle of adjuvant PBCT has been implemented.[12,22]

By total TC treatment, the study population was categorized into four groups: Surgery only (n=1405; 25%), PBCT (n=2521; 44%), RT (n=1550; 27%), and both PBCT and RT (PBCT+RT, n=231; 4.0%) (Table 1).

Statistical Methods

Median, interquartile range (IQR) and range were used to present continuous variables, and absolute and relative frequencies were used to present categorical variables.

Follow-up was defined as time from TC diagnosis date, until non-TC death date or censoring (date of TC-death, emigration or December 31, 2018, whichever occurred first). By splitting follow-up time at exact treatment dates for each treatment modality, treatment was analyzed as a time-varying covariate for all analyses to avoid immortal time bias. Total TC treatment burden, including relapse treatment, was included in the models.

Standardized mortality ratios (SMRs) were calculated by dividing the observed number of deaths in the study cohort by the expected number of deaths, calculated using mortality rates in the general Norwegian male population, matched by 5-year age groups and calendar year of follow-up. Absolute excess risks (AERs) were calculated using the following formula: [(observed number of deaths – expected number of deaths)/person-years of observation]*10000. Causes of deaths were classified according to the European Shortlist (Supplemental Table 1).[23] SMRs were calculated for total non-TC mortality, and according to specified non-TC SCs and groups of non-cancer causes of death if >4 deaths were observed, and stratified by follow-up time, age at diagnosis and attained age. For cause-specific analyses, all individuals with other causes of death not explicitly analyzed were censored at the date of death. The estimates with 95% confidence intervals (CIs) are presented for the total cohort and stratified on treatment modality.

The cumulative incidence of non-TC mortality was estimated using the Aalen-Johansen estimator treating TC death as a competing risk and presented with 95% CIs.[24]

The impact of treatment and histology on non-TC and SC mortality were investigated using Cox regression adjusting for age in addition to histology and treatment. As the proportional hazards assumption, assessed by Schoenfeld residuals, was violated for several of the analyses, both histology and treatment were analyzed as time-varying coefficients, i.e., by including an interaction between the covariates and follow-up time. In this respect we dichotomized follow-up time as up-to and after 10 years. The 10-year cut-off was chosen based on previous research which shows that non-TC mortality increases after 10 years.[2,15,16] Accordingly, we present hazard ratios (HRs) with corresponding 95% CIs for >10 years follow-up time unless otherwise specified. Non-TC mortality stratified by treatment groups and number of cisplatin-based chemotherapy cycles were illustrated using Kaplan-Meier failure plots adjusted for age centered on mean, and unadjusted Kaplan-Meier curves including population expected risks calculated from population lifetables containing mortality rates stratified by 1-year age groups and calendar year.

Data were analyzed using Stata statistical software (version MP 16.1; STATA, College Station, TX). A p-value <0.05 was considered statistically significant.

Results

Patient characteristics

The median follow-up time was 18.7 years (IQR 12.7-35.0), with follow-up time >20 years for 46% of the 5707 study participants (Table 1). Median age at diagnosis was 33.1 years (IQR 27.2-40.8), and histology was equally distributed with 52% seminoma and 48% nonseminoma.

Overall, 846 (15%) participants died during follow-up (Supplemental Table 1). TC was the cause of death for 181 (3.2%) men, of which 82% died within the first 5 years after diagnosis. Non-TC deaths were registered for 665 (12%) men, for which median follow-up time was 17.7 years (IQR 10.3-24.7), median age at diagnosis was 44.6 years (IQR 34.9-54.7), and 67% had seminoma histology (Table 1).

Total non-TC mortality

The overall 25-year crude cumulative non-TC mortality was 13.7% (95% CI 12.5-14.9), whereas the population expected (PE) risk was 11.3%. The 25-year cumulative non-TC mortality was 10.1 % after surgery (95% CI 8.0-12.4) (PE risk 10.6%), 9.5% after PBCT (95% CI 7.9-11.3) (PE risk 8.1%), 19.0% after RT (95% CI 16.8-21.2) (PE risk 14.9%), and 18.4% after PBCT+RT (95% CI 13.3-24.2) (PE risk 14.1%) (Supplemental Figure S2).

Compared with the general population, the overall non-TC mortality in the study cohort was significantly increased (SMR 1.23, 95% CI 1.14-1.33) (Table 2). No excess mortality appeared after treatment with surgery, while mortality after treatment with PBCT, RT or PBCT+RT was 1.23 to 2.04-fold higher than expected.

SMRs due to non-TC mortality increased significantly with increasing follow-up time ≥10 years after TC diagnosis (Table 2). After RT, increased SMRs were observed after the first post-diagnosis decade, while two decades passed before non-TC mortality increased following PBCT.

The highest non-TC mortality risk was observed in those <20 years at TC diagnosis (SMR 2.27, 95% CI 1.32-3.90), particularly in those treated with PBCT (SMR 2.49, 95% CI 1.29-4.78). The SMRs decreased with increasing age at diagnosis.

SC mortality

Non-TC SC was the cause of death in 257 (4.5%) of the study participants (Table 3), which constituted a 53% excess compared with the general population (SMR 1.53, 95% CI 1.35-173). Significantly 1.43 to 3.24-fold increased SC mortality emerged after treatment with PBCT, RT and PBCT+RT, while no increase followed treatment with surgery alone.

Analyses of death due to specified SCs unveiled overall 2.09 to 4.17-fold excesses caused by multiple cancers (Table 3). PBCT was associated with 1.69 to 6.82-fold excess mortality due to cancers of the lip/oral cavity/pharynx, esophagus, lung, bladder, and leukemia. After RT, 3.02 to 4.91-fold excess mortality emerged for cancers of the lip/oral cavity/pharynx, stomach, liver, pancreas and bladder.

With increasing age at TC diagnosis, a decrease in SMRs for SC mortality appeared; from SMR 3.68 (95% CI 1.19-11.40) in those <20 years, and SMR 2.00 (95% CI 1.28-2.99) in those 20-30 years, to SMR 1.28 (95% CI 1.04-1.57) in those >50 years.

Non-cancer mortality

Overall, 408 (7.1%) of the participants died as a result of non-cancer causes, which was 15% higher than among the general population (SMR 1.15, 95% CI 1.04-1.27) (Table 4). After treatment with PBCT, RT and PBCT+RT, the overall non-cancer mortality was 1.17 to 1.55-fold higher than expected.

Deaths due to infectious diseases were 3.73-fold increased after surgery (Table 4). PBCT was associated with 3.29-fold increased mortality due to diseases of the genitourinary

system and a 1.65-fold excess of suicide compared with the general population. RT was associated with a 2.46-fold increased risk of death due to diseases of the digestive system.

Apart from the increased mortality due to other heart diseases observed after PBCT+RT (Table 4), the overall CVD death was not increased in the total study cohort nor according to treatment modality. However, CVD death within the first year of follow-up was significantly increased after PBCT (SMR 3.90, 95% CI 1.26-12.08, AER 10.42, n=3; two acute myocardial infarctions, one stroke).

HRs for total non-TC mortality and SC mortality

Compared with surgery, total non-TC mortality was 1.42 to 2.79-fold increased after treatment with PBCT, RT and PBCT+RT (Table 5, Figure 1A). The risk was more pronounced for SC mortality, with 1.69 to 3.95-fold excess risks. In the multivariable models including histology, the hazards for PBCT remained unchanged, while minimal changes of hazards were observed for RT and PBCT+RT.

Non-TC mortality significantly increased after 4 (HR 1.41, 95% CI 1.01-1.99) and >4 (HR 2.04, 95% CI 1.25-3.35) cisplatin-based chemotherapy cycles, compared with surgery (Table 5, Figure 1B). Hazards for SC mortality were significantly 1.79 to 2.85-fold increased after 4 and >4 cycles, respectively.

RT with the L-field technique or paraaortic RT was associated with 1.48- to 1.60-fold increased non-TC mortality, and with 1.75 to 2.81-fold increased SC mortality, respectively, compared with surgery (Table 5). Significantly increased risks emerged after RT doses of ≥30 Gy for non-TC mortality, and after ≥20 Gy for SC mortality.

Discussion

We observed an excess total non-TC mortality, and in particular SC mortality, following treatment with PBCT or RT, but not after surgery alone when compared with the general population. The highest mortality risk was observed in the youngest TCS. This is, to the best of our knowledge, the first time the impact of number of cisplatin-based chemotherapy cycles on non-TC mortality was investigated in a population-based cohort with detailed TC treatment information. Significantly increased suicide risk emerged in the PBCT-group compared with the general population.

The overall 23% excess of non-TC mortality demonstrated in this study agrees with a previous Norwegian study,[3] and a recent publication by Sung et al.,[25] but lower than the 40% excess non-TC mortality reported in a previous Dutch study.[16] Corroborating previous studies, the non-TC mortality increased with follow-up time, especially beyond 20 years after TC diagnosis.[2,16] This demonstrates that adequate follow-up time is essential when studying mortality in TCS. In our study, the median follow-up time was longer for RT, and this may in part explain some of the differences in the observed risk estimates of PBCT and RT.

In line with previous publications,[3,16,25] we observed increased mortality caused by SCs for the total study cohort. In agreement with the Dutch study,[16] a distinct age-gradient emerged with decreasing risk of SC mortality with increasing age at TC diagnosis. Young age at TC diagnosis has been associated with an elevated SC incidence in previous studies. [8,13]

The 43% excess SC mortality emerging after PBCT is in line with a Danish study,[9] and lower than the SMR of 2.5 presented by the Dutch study.[16] Active compounds of cisplatin have been detected in plasma for up to 20 years after treatment,[26] and numerous long-term toxicities, including cisplatin-induced carcinogenesis, may follow this treatment.[27,28] After PBCT, we found a 6-fold increased SMR of bladder cancer, in contrast to the Dutch study.[16]

However, increased bladder cancer incidence after PBCT has been reported in multiple studies.[8-11] The renal clearance of cisplatin and the detection of cisplatin in urine up to 16 years after treatment, substantiates this result.[29]

In line with the Dutch study,[16] increased lung cancer death emerged after PBCT, while no increased risk followed surgery. The same pattern has been reported in other studies of lung cancer incidence after TC treatment.[8-11] Consistent with available literature, we found that mortality due to leukemia was 3-fold increased after PBCT compared with the general population, however based on few events.[16] Increased leukemia mortality in TCS has also been reported in register-based studies, though without treatment details.[3,25]

We report increased non-TC mortality after >2 cisplatin-based chemotherapy cycles, with significant hazards observed after >3 cycles. The even stronger association that emerged for SC mortality is in line with the Dutch study,[16] although that study lacked complete treatment data. Statistical significance was not reached for 3 cycles, probably because of few numbers and a shorter follow-up time. Compared with surgery, we did not find an indication of increased mortality after one or two courses of adjuvant chemotherapy, however follow-up time was shorter than for >3 cycles.

Consistent with previous studies,[9,16-19] we found an overall 60% excess SC mortality after RT compared with the general population. As previously described,[16,18,19] significantly elevated SMRs emerged for cancers within the boundaries of the prior RT field (cancers of the stomach, liver, pancreas and bladder), confirming the previously reported dose-dependency.[16] Because the use of adjuvant RT was discarded during the early 2000s,[12] we expect the malignancies and mortality associated with RT to persist for the next decade, before gradually diminishing.

The overall 15% excess risk of non-cancer death is in line with previous publications.[15,16] The 23% and 55% excess non-cancer mortality after PBCT and PBCT+RT, respectively, are comparable with one previous report,[15] whereas two previous studies reported higher estimates for non-cancer mortality after PBCT.[6,16] The increased mortality due to infections after surgery is in line with a previous study.[15]

Based on available literature, there is a concern that TCS are more liable to committing suicide,[3,18,30,31] and in the present study increased suicide risk emerged after PBCT compared with the general population. A population-based study from Canada recently reported that the use of mental health services were more common in TCS, especially in TCS with treatment beyond surveillance.[32] Cisplatin-based chemotherapy is associated with numerous side-effects like fatigue, neuropathy and hypogonadism, long-term cognitive impairment and memory problems.[33,34] Increased risk of anxiety, but not depression, has been observed among long-term TCS, especially in the youngest.[35] A review identified passive coping strategies and treatment-related side effects in TCS as associated with an inferior psychological outcome.[36] Together, these conditions may endanger TCS, and in particular the youngest TC patients treated with cisplatin in a vulnerable life period, for an increased suicide risk.[37] This calls for health professionals´ increased awareness towards suicide risk factors in TCS in order to prevent future deaths from suicide.

PBCT is associated with an increased long-term risk of CVD.[5,7] While some studies have found an association between PBCT and long-term CVD mortality,[5,16] other studies, including the present one, failed to support this association.[3,6] The most likely explanation for the lack of an association between PBCT and CVD mortality in the present study is the general reduction of coronary heart disease (CHD) mortality during the last two decades, achieved by a reduction in modifiable CVD risk factors combined with improvement in treatment options for CHD.[38,39] Additionally, based on increasing knowledge regarding excess

CVD morbidity after PBCT,[40] screening for CVD risk factors was gradually implemented in the TC follow-up guidelines in Norway from 2007.[41]

It is hypothesized that cytotoxic therapy induces an ageing phenotype that imperils TCS of early onset CVD or SCs,[42-45] or leads to epigenetic changes in genes associated with metabolic syndrome.[46] Unfavorable lifestyle factors (smoking, physical inactivity, unhealthy nutrition, alcohol abuse), may contribute to premature cellular senescence and increased SC risk.[45] Continued tobacco use after a first cancer has in numerous studies been linked to increased SC risk.[47] In a recent report,[25] smoking-related cancers (lung, bladder, oral cavity/pharynx and esophagus) accounted for up to 45% of the total SC mortality. Our results, with increased SC mortality due to smoking- and alcohol-related cancers, support such observations.

Strengths of the present study include complete information on treatment burden in an unselected, nationwide cohort with extended follow-up time. Incident TC cases were provided by the CRN, a cancer registry with very high completeness.[20] Another strength is the near-complete coverage of the NCoDR.[48]

A limitation is the lack of information on possible lifestyle risk factors for cancer and CVD. Further, when calculating SMRs of total SC, TC deaths were not excluded from the background data provided by the NCoDR, leading to a potential underestimation of the SMRs. However, since TC deaths constitutes a small fraction of the total, this bias is considered negligible. When conducting multiple hypothesis testing, the risk of type 1 errors increases. As advised by Rothman,[49] correcting for multiple testing was not done due to the risk of increasing type II errors. Caution must therefore be taken when interpreting results involving only a few cases.

In conclusion, TCS treated with PBCT or RT suffer increased mortality rates compared with the general population. The most notable excess mortality was caused by non-TC SCs, and measures to avoid delayed SC diagnosis is essential. We hypothesize that cytotoxic treatment is the main risk factor for increased mortality. The increased mortality risk might be reduced by lifestyle improvements, which should be recommended following TC treatment. It is of outmost importance that TCS and health personnel involved in the follow-up are aware of the increased risk of premature mortality.

Figure Legends

Figure 1

Non-TC mortality by follow-up time adjusted for age centered on mean. A) Stratified by treatment group, B) Stratified by number of cisplatin-based chemotherapy cycles. The risk tables present the crude number of individuals by follow-up time.

Abbreviations: TC, testicular cancer; PBCT, platinum-based chemotherapy; RT, radiotherapy

Supplemental Figure S2

Crude non-TC mortality by follow-up time, stratified by treatment group. The dashed lines represent population expected risks. The risk table present number of individuals by follow-up time.

Abbreviations: TC, testicular cancer; PBCT, platinum-based chemotherapy; RT, radiotherapy

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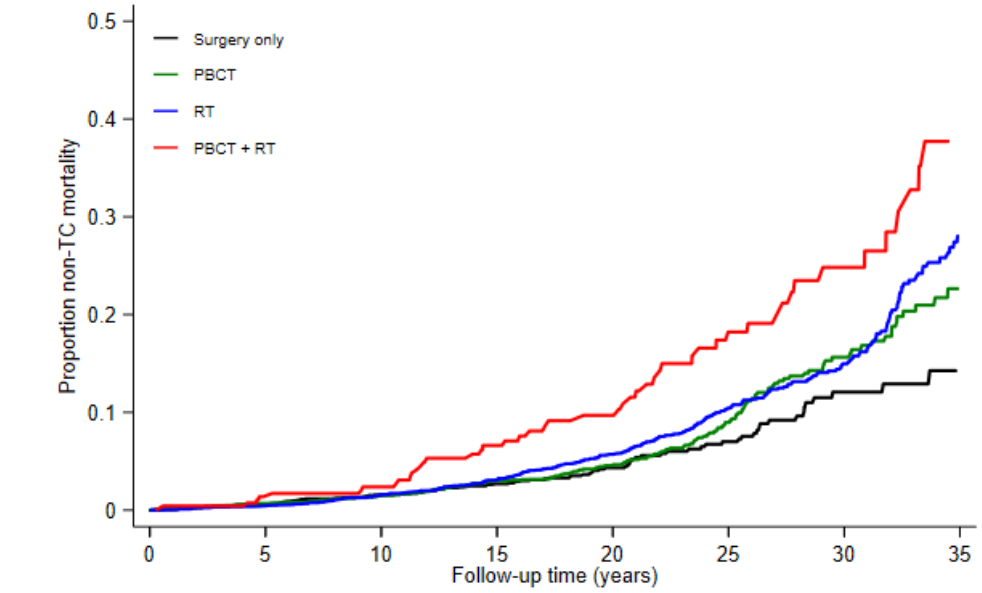
Figure 1.

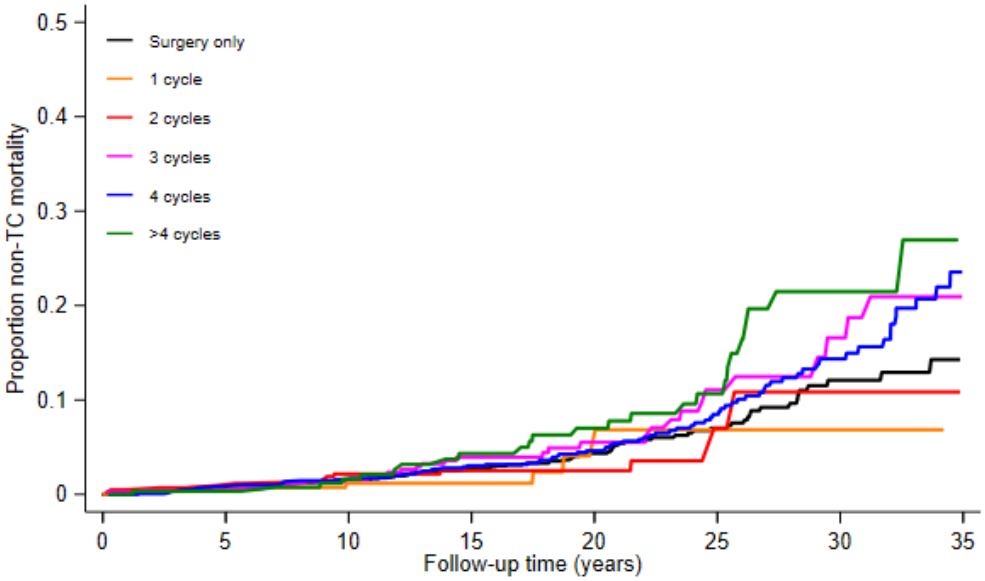
Figure 1A)



N at risk:

Surgery only	5611	1413	1264	842	499	322	172	65
PBCT	91	2391	2169	1416	953	559	304	113
RT	5	1541	1478	1334	1023	639	318	91
PBCT + RT	0	130	138	123	98	69	37	14

Figure 1B



N at risk:

Surgery only	5611	1413	1264	842	499	322	172	65
1 cycle	5	212	172	86	20	5	4	2
2 cycles	3	307	290	198	112	35	19	1
3 cycles	2	419	384	246	153	102	71	28
4 cycles	46	914	873	657	496	313	184	69
>4 cycles	33	221	204	168	135	82	24	12

Table 1. Patient Characteristics

Characteristic	Total Study Cohort (n = 5707)	Non-TC Death (n = 665, 12%)
Decade of first TC diagnosis		
1980-1989	1303 (23)	342 (51)
1990-1999	1919 (34)	225 (34)
2000-2009	2485 (43)	98 (15)
Follow-up, y, median (IQR)[a] (range)	18.7 (12.7-35.0) (0.0-39.0)	17.7 (10.3-24.7) (0.0-38.2)
Follow-up groups		
<1	76 (1.3)	12 (1.8)
1-10	582 (10)	153 (23)
10-20	2476 (43)	214 (32)
20-30	1742 (31)	211 (32)
>30	831 (15)	75 (11)
Age at diagnosis, y, median (IQR) (range)	33.1 (27.2-40.8) (16.0-83.8)	44.6 (34.9-54.7) (16.5-83.8)
Age groups, y		
<20	219 (3.8)	13 (2.0)
20-30	1931 (34)	81 (12)
30-40	2012 (35)	160 (24)
40-50	979 (17)	176 (27)
>50	566 (10)	235 (35)
Histology		
Seminoma	2976 (52)	447 (67)
Nonseminoma	2731 (48)	218 (43)
Age at diagnosis according to histology, y, median (IQR) (range)		
Seminoma	36.8 (31.0-44.6) (17.3-83.8)	46.6 (38.2-56.3) (18.8-83.8)
Nonseminoma	28.9 (24.2-35.5) (16.0-78.5)	36.8 (28.6-49.8) (16.5-78.5)
Initial disease stage[b]		
I	3996 (70)	456 (69)
Mk+/II	1144 (20)	140 (21)
III	119 (2.1)	27 (4.1)
IV	448 (7.9)	42 (6.3)
Treatment[c]		
Surgery only[d]	1405 (25)	109 (16)
PBCT	2521 (44)	184 (28)
RT	1550 (27)	323 (49)
PBCT + RT	231 (4.0)	49 (7.4)
Cause of first-line chemotherapy		
Adjuvant, CS I	883 (32)	28 (12)
Primary metastatic disease	1580 (57)	175 (75)
Recurrence	290 (11)	30 (13)
First chemotherapy regimen		
BEP-20	1553 (56)	88 (38)
CVB	377 (14)	77 (33)
EP	254 (9.3)	24 (11)
Other cisplatin-based CT[e]	187 (6.8)	21 (9.0)
Adjuvant Carboplatin	315 (11)	8 (3.4)
CEB	44 (1.6)	11 (4.7)
Other[f]	22 (0.8)	4 (1.7)
Cumulative no. of cisplatin-based CT cycles[g]		
1	242 (10)	6 (2.8)
2	325 (14)	15 (7.1)
3	457 (19)	34 (16)
4	1038 (43)	124 (59)
> 4	337 (14)	32 (15)
RT first field		
L-field[h]	1408 (79)	315 (85)
Paraaortal	275 (15)	39 (11)
Supradiafragmatic	13 (0.7)	1 (0.3)
Supra- and infradiafragmatic[i]	21 (1.2)	11 (3.0)
Other[j]	64 (3.6)	6 (1.6)
RT dose for first RT field		
1-20 Gy	13 (0.7)	1 (0.3)
20-29Gy	522 (29)	54 (15)
30-39 Gy	1005 (56)	232 (62)
≥40 Gy	241 (14)	85 (23)

Note: Data are presented as n (%), unless otherwise stated.

Abbreviations: TC, testicular cancer; n, number; y, years; IQR, interquartile range; Mk+, marker positive; PBCT, platinum-based chemotherapy; RT, radiotherapy; PBCT + RT, combination of PBCT and RT; CS I, clinical stage I; BEP-20, bleomycin, etoposide and cisplatin; CVB, cisplatin, vinblastine and bleomycin; EP, etoposide and cisplatin; CEB, carboplatin, etoposide and bleomycin; CT, chemotherapy; no, number; Gy, grey.

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[a] Follow-up until death of all causes, emigration or December 31st 2018, whichever occurred first.
[b] As described by Peckham et al.[21]
[c] Based on total treatment burden.
[d] The surgery only group included men followed with surveillance after orchiectomy (n = 1167; 21%) and men who underwent additional retroperitoneal lymph node dissection without PBCT or RT (n = 250; 4.4%). Also included in this group are 4 men who were diagnosed with clinical stage IV but for various reasons did not receive further treatment.
[e] Of which a total of 143 were dose-escalated cisplatin-based chemotherapy.
[f] Carboplatin monotherapy in metastatic setting (n=17), sendoxan/adriamycin (n=1), CAOS (actinomycin D, adriamycin, vincristine, sendoxan) (n=3), actinomycin D (n=1).
[g] Only cisplatinbased chemotherapy cycles are accounted for in cumulative number. A total of 350 men received carboplatin-based chemotherapy (adjuvant carboplatin monotherapy n=299, other carboplatin-based chemotherapy (n=52)). In total two men received non-PBCT (actinomycin D (n=1), CAOS (actinomycin D, adriamycin, vincristine, sendoxan) (n=1)).
[h] L-field or dogleg-field. Included in this category are also 55 individuals who received RT of groin in addition to L-field and 2 individuals who received a reversed Y-field.
[i] 16 of 21 individuals received infradiafragmatic RT as first RT-field and a short while later received supradiafragmatic RT.
[j] RT towards bone (n=21), CNS (n=21), abdominal residual masses (n=16), intraoperative RT (n=1), skin lesions (n=1), non-specified sites (n=4)

Table 2. Total Non-TC Mortality by Follow-up Time, Age at Diagnosis and Attained Age According to Treatment Group

Variable	Total cohort			Surgery			PBCT			RT			PBCT + RT		
	n	**SMR (95% CI)**	**AER**	**n**	**SMR (95% CI)**	**AER**	**n**	**SMR (95% CI)**	**AER**	**n**	**SMR (95% CI)**	**AER**	**n**	**SMR (95% CI)**	**AER**
Total non-TC death	665	**1.23 (1.14-1.33)**	11.14	109	0.95 (0.79-1.14)	-2.21	184	**1.23 (1.07-1.43)**	7.68	323	**1.28 (1.15-1.43)**	19.55	49	**2.04 (1.54-2.70)**	75.45
Follow-up time															
<1 year	12	0.84 (0.48-1.48)	-3.94	3	0.64 (0.21-1.98)	-9.07	5	1.29 (0.54-3.10)	5.23	3	0.55 (0.18-1.71)	-15.26	1	4.48 (0.63-31.80)	129.68
>1-10 years	153	1.01 (0.86-1.18)	0.20	42	1.08 (0.80-1.46)	2.48	48	1.02 (0.77-1.35)	0.41	57	0.94 (0.77-1.35)	-2.66	6	1.12 (0.50-2.49)	5.15
10-20 years	214	**1.15 (1.01-1.32)**	7.48	35	0.86 (0.62-1.20)	-6.70	53	1.04 (0.80-1.37)	1.48	109	**1.25 (1.04-1.51)**	16.87	17	**2.29 (1.42-3.69)**	79.65
20-30 years	211	**1.48 (1.29-1.69)**	42.00	26	1.11 (0.76-1.63	8.04	60	**1.68 (1.30-2.16)**	41.43	107	**1.41 (1.17-1.70)**	47.73	18	**2.37 (1.49-3.76)**	154.89
30-40 years	75	**1.64 (1.31-2.06)**	89.51	3	0.41 (0.13-1.27)	-61.53	18	1.55 (0.98-2.46)	50.89	47	**2.01 (1.51-2.67)**	203.24	7	2.05 (0.98-4.31)	234.21
Age at diagnosis															
<20 years	13	**2.27 (1.32-3.90)**	14.42	3	2.06 (0.66-6.34)	11.78	9	**2.49 (1.29-4.78)**	16.55	0	0	-13.71	1	4.52 (0.64-32.06)	50.97
20-30 years	81	**1.26 (1.01-1.56)**	4.06	14	0.84 (0.50-1.42)	-2.34	42	**1.42 (1.05-1.92)**	6.28	21	1.29 (0.84-1.99)	5.47	4	1.94 (0.73-5.17)	18.88
30-40 years	160	**1.36 (1.16-1.58)**	10.58	22	0.93 (0.62-1.42)	-1.75	45	1.22 (0.91-1.64)	5.67	81	**1.53 (1.23-1.90)**	18.44	12	**2.57 (1.46-4.53)**	61.40
40-50 years	176	**1.42 (1.23-1.65)**	29.47	20	1.05 (0.67-1.63)	2.59	41	**1.28 (0.94-1.74)**	15.99	95	**1.43 (1.17-1.75)**	36.80	20	**3.04 (1.96-4.71)**	208.58
>50 years	235	1.03 (0.91-1.17)	7.58	50	0.92 (0.70-1.22)	-20.79	47	1.00 (0.75-1.33)	-0.56	126	1.08 (0.91-1.29)	23.40	12	1.14 (0.65-2.01)	51.44
Attained age															
<40 years	51	1.21 (0.92-1.59)	2.25	12	1.04 (0.59-1.84)	0.44	26	1.29 (0.88-1.90)	3.00	12	1.26 (0.72-2.22)	3.13	1	1.04 (0.15-7.41)	0.48
40-60 years	227	**1.31 (1.15-1.49)**	9.26	45	1.25 (0.94-1.68)	7.06	82	**1.37 (1.10-1.70)**	10.03	84	1.17 (0.95-1.45)	6.02	16	**2.46 (1.51-4.01)**	51.53
60-75 years	235	**1.25 (1.10-1-42)**	36.68	20	0.57 (0.37-0.88)	-63.11	47	1.06 (0.79-1.40)	7.47	143	**1.44 (1.22-1.69)**	67.42	25	**2.76 (1.87-4.09)**	295.23
>75 years	152	1.12 (0.95-1.31)	77.77	32	0.99 (0.70-1.40)	-6.73	29	1.18 (0.82-1.70)	112.62	84	1.17 (0.94-1.45)	112.56	7	0.93 (0.44-1.95)	-51.54

Note: Significant associations for SMRs are marked with bold.

Abbreviations: TC, testicular cancer; PBCT, platinum-based chemotherapy; RT, radiotherapy; n, number; SMR, standardized mortality ratio; 95% CI, 95% confidence interval, AER, absolute excess risk.

Table 3. Risk of Deaths Due to Non-TC Second Cancers (if >4 Deaths Observed) According to Treatment Groups

Cause of Death	Total			Surgery			PBCT			RT			PBCT + RT			EU code[i]
	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	
Total non-TC SC	257	**1.53 (1.35-1.73)**	7.94	39	1.13 (0.83-1.55)	1.71	64	**1.43 (1.12-1.83)**	4.25	130	**1.59 (1.34-1.89)**	13.38	24	**3.24 (2.17-4.83)**	50.13	2.1-TC
Specified cancers																
Oral cavity, pharynx[ii]	9	**3.89 (2.03-7.48)**	0.60	0	0	-0.17	4	**6.82 (2.56-18.16)**	0.75	5	**4.28 (1.78-10.27)**	1.06	0	0	-0.30	2.1.1
Esophagus	7	**2.29 (1.09-4.80)**	0.35	3	**4.83 (1.56-14.97)**	0.88	3	**3.74 (1.21-11.59)**	0.48	1	0.66 (0.09-4.69)	-0.14	0	0	-0.36	2.1.2
Stomach	18	**2.92 (1.84-4.63)**	1.06	3	2.45 (0.79-7.59)	0.66	1	0.69 (0.10-4.90)	-0.10	10	**3.15 (1.69-5.85)**	1.89	4	**12.85 (4.82-34.23)**	11.15	2.1.3
Colorectal[iii]	29	1.31 (0.91-1.88)	0.61	3	0.66 (0.21-2.06)	-0.56	10	1.73 (0.93-3.22)	0.93	15	1.38 (0.83-2.28)	1.13	1	1.01 (0.14-7.14)	0.03	2.1.4
Liver[iv]	5	1.75 (0.73-4.20)	0.19	1	1.67 (0.24-11.85)	0.15	0	0	-0.18	4	**3.02 (1.13-8.04)**	0.74	0	0	-0.33	2.1.5
Pancreas	33	**3.20 (2.28-4.51)**	2.03	5	2.40 (1.00[v]-5.78)	1.08	3	1.10 (0.35-3.40)	0.06	22	**4.36 (2.87-6.62)**	4.70	3	**6.86 (2.21-21.27)**	7.74	2.1.6
Lung[vi]	49	1.26 (0.95-1.66)	0.89	3	0.39 (0.13-1.21)	-1.73	17	**1.69 (1.05-2.72)**	1.53	25	1.28 (0.86-1.89)	1.23	4	2.30 (0.86-6.13)	6.83	2.1.8
Melanoma of skin	8	1.38 (0.69-2.77)	0.20	3	2.52 (0.81-7.82)	0.67	1	0.59 (0.08-4.21)	-0.15	3	1.12 (0.36-3.47)	0.09	1	4.43 (0.62-31.43)	2.33	2.1.9
Prostate	14	0.78 (0.46-1.32)	-0.35	1	0.26 (0.04-1.88)	-1.03	3	0.79 (0.25-2.45)	-0.18	7	0.74 (0.35-1.55)	-0.68	3	**3.27 (1.06-10.15)**	6.29	2.1.14
Kidney	5	1.26 (0.53-3.03)	0.09	2	2.53 (0.63-10.14)	0.45	1	1.03 (0.15-7.34)	0.01	2	0.99 (0.25-3.95)	-0.01	0	0	-0.54	2.1.15
Bladder	16	**4.17 (2.56-6.81)**	1.09	1	1.23 (0.17-8.75)	0.07	5	**6.33 (2.63-15.21)**	0.93	10	**4.91 (2.64-9.13)**	2.20	0	0	-0.60	2.1.16
Brain and CNS	10	1.32 (0.71-2.45)	0.22	3	1.89 (0.61-5.86)	0.52	5	2.07 (0.86-4.96)	0.57	2	0.61 (0.15-2.42)	-0.36	0	0	-0.85	2.1.17
Lymphoma[vii]	5	1.12 (0.46-2.68)	0.05	3	**3.45 (1.11-10.70)**	0.79	0	0	-0.24	1	0.43 (0.06-3.05)	-0.37	1	4.83 (0.68-34.30)	2.39	2.1.19
Leukemia	8	**2.09 (1.05-4.19)**	0.37	0	0	-0.29	3[viii]	**3.26 (1.05-10.12)**	0.46	4	2.04 (0.77-5.44)	0.57	1	5.74 (0.81-40.76)	2.51	2.1.20

Note: Significant associations for SMRs are marked with bold.

Abbreviations: TC, testicular cancer; PBCT, platinum-based chemotherapy; RT, radiotherapy; SMR, standardized mortality ratio; n, number; 95% CI, 95% confidence interval; AER, absolute excess risk.

[i] Eurostat. European Shortlist of Causes of Death, May 2012. Available from: http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=COD_2012&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC.

[ii] Lip, oral cavity and pharynx
[iii] Colon, rectum and anus
[iv] Liver and intrahepatic bile ducts
[v] 0.997
[vi] Trachea, bronchus and lung
[vii] Hodgkin disease and lymphomas
[viii] Time to leukemia death from PBCT: 2.6 years (4 cycles PBCT), 17.8 years (3 cycles PBCT) and 27.1 years (>4 cycles PBCT).

Table 4. Risk of Death Due to Selected Non-Cancer Causes According to Treatment Groups

Cause of Death	Total			Surgery			PBCT			RT			PBCT + RT			EU code [a]
	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	n	SMR (95% CI)	AER	
Non-cancer death, total	**408**	**1.15 (1.04-1.27)**	4.71	70	0.92 (0.72-1.16)	-2.40	120	**1.23 (1.03-1.47)**	4.95	193	**1.17 (1.01-1.34)**	7.68	25	**1.55 (1.05-2.30)**	26.86	
Infectious and parasitic diseases	15[b]	**2.35 (1.42-3.90)**	0.77	5	**3.73 (1.55-8.95)**	1.36	4	2.49 (0.94-6.64)	0.53	6	1.91 (0.86-4.25)	0.79	0	0	-0.88	1.
Endocrine and metabolic diseases[c]	12	1.13 (0.64-1.99)	0.12	2	0.91 (0.23-3.64)	-0.07	0	0	-0.63	9	1.76 (0.91-3.37)	1.07	1	2.16 (0.30-15.37)	1.63	4.
Mental and behavioural disorders	18	1.00 (0.63-1.59)	0.00	3	0.79 (0.26-2.45)	-0.29	2	0.38 (0.09-1.51)	-0.72	12	1.47 (0.84-2.59)	1.07	1	1.34 (0.19-9.49)	0.76	5.
Diseases of the nervous system[d]	20	1.16 (0.75-1.80)	0.25	5	1.38 (0.57-3.31)	0.51	6	1.27 (0.57-2.83)	0.28	8	0.98 (0.49-1.96)	-0.04	1	1.40 (0.20-9.91)	0.85	6.
Cardiovascular disease	151	1.01 (0.86-1.18)	0.08	28	0.89 (0.62-1.29)	-1.25	42	1.18 (0.87-1.60)	1.41	71	0.94 (0.75-1.19)	-1.23	10	1.29 (0.69-2.40)	6.83	7.
Ischemic heart diseases	90	1.12 (0.91-1.37)	0.84	20	1.22 (0.79-1.89)	1.34	22	1.16 (0.77-1.77)	0.68	45	1.10 (0.82-1.47)	1.10	3	0.70 (0.22-2.16)	-3.96	7.1
Other heart diseases	31	1.37 (0.97-1.95)	0.75	5	1.03 (0.43-2.47)	0.05	9	1.76 (0.91-3.34)	0.85	11	0.96 (0.53-1.73)	-0.13	6[e]	**5.30 (2.38-11.81)**	14.72	7.2
Cerebrovascular diseases	17	0.65 (0.40-1.05)	-0.82	1	0.18 (0.03-1.27)	-1.71	6	1.04 (0.47-2.31)	0.05	9	0.67 (0.35-1.29)	-1.22	1	0.73 (0.10-5.20)	-1.09	7.3
Other circulatory diseases	13	0.85 (0.49-1.46)	-0.21	2	0.63 (0.16-2.52)	-0.44	5	1.45 (0.60-3.48)	0.34	6	0.76 (0.34-1.69)	-0.53	0	0	-2.36	7.4
Diseases of the respiratory system	33	0.96 (0.68-1.35)	-0.13	2	0.27 (0.07-1.09)	-1.99	10	1.29 (0.70-2.40)	0.50	18	1.02 (0.64-1.62)	0.10	3	1.78 (0.57-5.53)	3.99	8.
Diseases of the digestive system	32	**1.89 (1.34-2.67)**	1.35	4	1.15 (0.43-3.089	0.20	6	1.31 (0.59-2.91)	0.31	20	**2.46 (1.59-3.82)**	3.29	2	2.68 (0.67-10.71)	3.78	9.
Diseases of the genitourinary system	7	1.55 (0.74-3.24)	0.22	2	1.96 (0.49-7.85)	0.36	3[f]	**3.29 (1.06-10.21)**	0.46	1	0.42 (0.06-3.02)	-0.37	1	4.07 (0.57-38.89)	2.27	12.
External causes	85	**1.25 (1.01-1.55)**	1.52	14	0.87 (0.51-1.46)	-0.79	35	1.34 (0.96-1.86)	1.95	32	1.36 (0.96-1.92)	2.34	4	1.79 (0.67-4.78)	5.35	17.
Accidents	51	1.22 (0.93-1.61)	0.83	9	0.91 (0.48-1.76)	-0.32	19	1.22 (0.78-1.92)	0.76	21	1.41 (0.92-2.16)	1.68	2	1.41 (0.35-5.64)	1.75	17.1
Suicide	33	1.38 (0.98-1.94)	0.81	4	0.70 (0.26-1.86)	-0.65	16	**1.65 (1.01-2.69)**	1.39	11	1.41 (0.78-2.55)	0.89	2	2.73 (0.68-10.93)	3.84	17.2

Note: The table present results for those main groups of causes of death (as defined by EU-codes) if >4 deaths observed. Significant associations for SMRs are marked with bold.

Abbreviations: PBCT, platinum-based chemotherapy; RT, radiotherapy; n, number; SMR, standardized mortality ratio; 95% CI, 95% confidence interval; AER, absolute excess risk.

[a] Eurostat. European Shortlist of Causes of Death, May 2012. Available from: http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=COD_2012&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC.

[b] Of which AIDS = 3
[c] Endocrine, nutritional and metabolic diseases
[d] Diseases of the nervous system and the sense organs
[e] Two participants received supradiaphragmatic RT. The following diseases were listed as cause of death: Endocarditis=1, mitral valve disease=1, aortic valve disease = 2, unspecified heart disease = 1, cardiomyopathy=1.
[f] Kidney failure (n=1), urinary tract infection (n=1), prostatitis (n=1).

Table 5. HRs for Total Non-TC Mortality and Non-TC SC Mortality According to Treatment Groups, Treatment Intensity and Histology After >10 Years of Follow-up

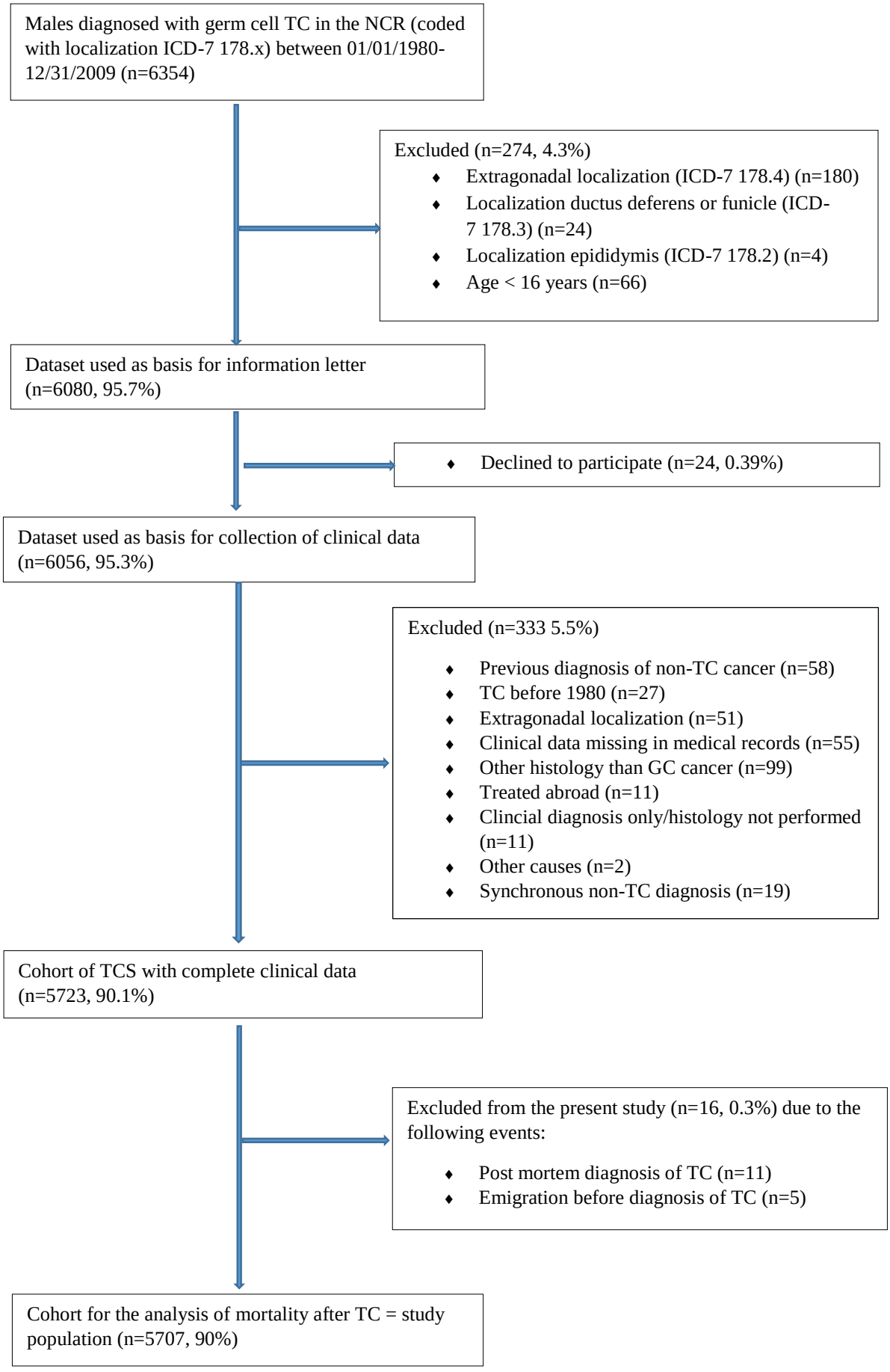
	All non-TC mortality		Non-TC SC mortality		Follow-up, y, median (IQR)
Variable	Age-adjusted HR (95% CI)	Multivariable HR (95% CI)	Age-adjusted HR (95% CI)	Multivariable HR (95% CI)	
Treatment Groups					
Surgery	1 (ref)	1 (ref)	1 (ref)	1 (ref)	16.8 (12.1-24.0)
PBCT	**1.42 (1.05-1.91)**	**1.42 (1.05-1.91)**	**1.69 (1.05-2.73)**	**1.69 (1.05-2.73)**	16.8 (11.6-23.9)
RT	**1.61 (1.23-2.12)**	**1.94 (1.39-2.73)**	**1.87 (1.20-2.90)**	**1.83 (1.07-3.14)**	23.3 (17.7-28.8)
PBCT + RT	**2.79 (1.89-4.13)**	**3.27 (2.14-5.00)**	**3.95 (2.22-7.02)**	**3.89 (2.06-7.33)**	17.0 (3.7-26.3)
Cisplatin-based chemotherapy cycles[i]					
Surgery	1 (ref)	1 (ref)	1 (ref)	1 (ref)	
1	0.79 (0.25-2.53)	0.78 (0.25-2.49)	NA	NA	13.0 (9.9-17.0)
2	0.43 (0.16-1.19)	0.43 (0.15-1.17)	0.30 (0.04-2.20)	0.30 (0.04-2.21)	17.1 (13.5-22.3)
3	1.47 (0.91-2.39)	1.43 (0.88-2.33)	1.64 (0.76-3.54)	1.65 (0.76-3.56)	16.7 (11.7-24.2)
4	**1.41 (1.01-1.99)**	**1.43 (1.02-2.01)**	**1.79 (1.06-3.04)**	**1.79 (1.05-3.03)**	20.7 (14.0-28.1)
>4	**2.04 (1.25-3.35)**	**1.98 (1.21-3.25)**	**2.85 (1.40-5.84)**	**2.87 (1.40-5.88)**	18.4 (4.0-25.6)
Carboplatin, adjuvant	1.21 (0.38-3.86)	1.39 (0.43-4.52)	2.33 (0.55-9.97)	2.29 (0.52-10.05)	11.2 (10.0-12.8)
RT field					
Surgery	1 (ref)	1 (ref)	1 (ref)	1 (ref)	
L-field	**1.60 (1.21-2.12)**	**1.94 (1.38-2.73)**	**1.75 (1.12-2.74)**	1.71 (0.99-2.96)	24.4 (18.1-29.7)
Paraaortic	1.48 (0.89-2.44)	1.77 (1.03-3.03)	**2.81 (1.45-5.45)**	**2.75 (1.33-5.67)**	19.6 (16.3-23.0)
Supra & infradiafragmatic	**5.07 (2.04-12.63)**	**6.17 (2.42-15.70)**	**5.11 (1.20-21.68)**	**4.98 (1.13-21.91)**	12.5 (3.5-27.3)
RT dose for first abdominal RT-field					
Surgery	1 (ref)	1 (ref)	1 (ref)	1 (ref)	
1-20 Gy	1.75 (0.24-12.69)	2.05 (0.28-14.97)	NA	NA	18.5 (13.1-21.4)
20-29 Gy	1.31 (0.87-1.98)	1.55 (0.98-2.44)	**1.93 (1.06-3.52)**	1.84 (0.94-3.59)	19.5 (15.5-22.2)
30-39 Gy	**1.68 (1.26-2.23)**	**2.00 (1.41-2.85)**	**1.69 (1.06-2.70)**	1.60 (0.91-2.82)	25.6 (19.5-29.8)
≥40 Gy	**1.54 (1.04-2.28)**	**1.85 (1.18-2.88)**	**2.33 (1.32-4.11)**	**2.20 (1.14-4.24)**	32.4 (23.5-35.7)
Histology					
Nonseminoma	1 (ref)	1 (ref)	1 (ref)	1 (ref)	19.5 (12.9-26.9)
Seminoma	**1.23 (1.02-1.50)**	0.93 (0.71-1.23)	1.34 (1.00-1.80)[ii]	1.10 (0.71-1.69)	18.0 (12.6-25.1)

Note: The multivariable models investigating treatment variables were adjusted for histology in addition to age at TC diagnosis. The multivariable models investigating histology were adjusted for treatment groups in addition to age at TC diagnosis.

Abbreviations: HR, hazard ratio; TC, testicular cancer; SC, second cancer; y, years; 95% CI, 95% confidence interval; RT, radiotherapy; NA, not applicable; Gy, gray.

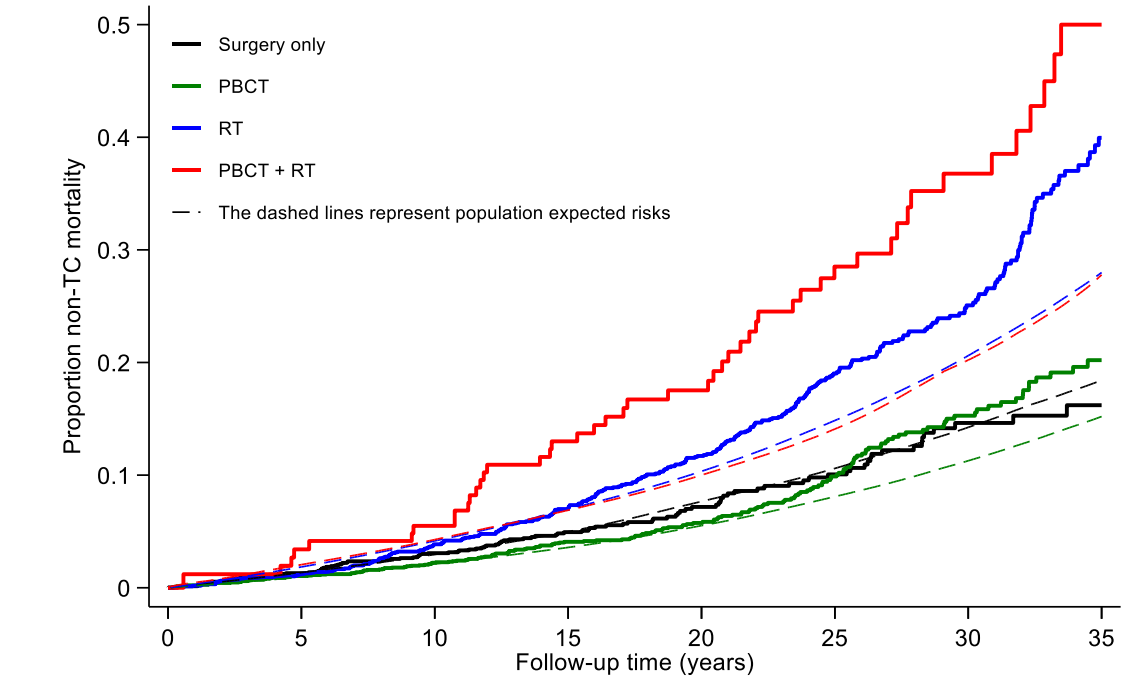
[i] Numbers 1 to >4 refers to number of cisplatin-based chemotherapy cycles. Carboplatin adjuvant refers to carboplatin monotherapy in adjuvant setting for stage I seminoma.
[ii] P-value= 0.054

Supplemental Figure S1. Flow Chart Presenting the Study Cohort



Abbreviations: TC, testicular cancer; NCR, the Norwegian Cancer Registry; ICD-7, International Classification of Diseases version 7, GC, germ cell; TCS, testicular cancer survivors.

Supplemental Figure S2



N at risk:								
Surgery only	5611	1413	1264	842	499	322	172	65
PBCT	91	2391	2169	1416	953	559	304	113
RT	5	1541	1478	1334	1023	639	318	91
PBCT + RT	0	130	138	123	98	69	37	14

Supplemental Table 1. All Causes of Death in the Study Cohort According to Time Since TC Diagnosis

Causes of Death	All	1 y	>1-10 y	>10-20 y	>20-30 y	>30 y	EU code[a]
Total	846	73	251	227	218	77	
TC	181	61	98	13	7	2	
Total excluding TC	665	12	153	214	211	75	
Non-TC second cancers, total	257	1	34	90	97	35	2.1 excl. TC
Lip, oral cavity, pharynx	9	0	1	4	4	0	2.1.1
Oesophagus	7	0	1	3	3	0	2.1.2
Stomach	18	0	2	11	3	2	2.1.3
Colon, rectum and anus	29	0	3	8	15	3	2.1.4
Liver and intrahepatic bile ducts	5	0	0	2	2	1	2.1.5
Pancreas	33	0	6	8	13	6	2.1.6
Larynx	2	0	0	0	2	0	2.1.7
Trachea, bronchus and lung	49	0	3	17	24	5	2.1.8
Melanoma of skin	8	0	1	5	2	0	2.1.9
Breast	1	0	0	0	1	0	2.1.10
Prostate	14	0	1	5	4	4	2.1.14
Kidney	5	0	1	1	2	1	2.1.15
Bladder	16	0	1	1	8	6	2.1.16
Brain and CNS	10	0	3	6	1	0	2.1.17
Hodgkin disease and lymphomas	5	0	2	2	1	0	2.1.19
Leukemia	8	0	4	2	2	0	2.1.20
Other malignant hematological neoplasms[b]	2	0	0	0	0	2	2.1.21
Other malignant neoplasms	36	1	5	15	10	5	2.1.22
Non-cancer death, total	408	11	119	124	114	40	
Infectious and parasitic diseases	15[c]	1	6	3	4	1	1.
Diseases of the blood and blood-forming organs[d]	2	0	0	1	0	1	3.
Endocrine, nutrional and metabolic diseases	12	0	0	6	4	2	4.
Mental and behavioural disorders	18	1	8	1	6	2	5.
Disease of the nervous system	20	0	11	3	5	1	6.

Cardiovascular disease	151	6	42	50	38	15	7.
Ischemic heart disease	90	4	26	29	23	8	7.1
Other heart diseases	31	1	7	11	9	3	7.2
Cerebrovascular diseases	17	1	8	6	1	1	7.3
Other circulatory	13	0	1	4	5	3	7.4
Diseases of the respiratory system	33	1	6	9	10	7	8.
Diseases of the digestive system	32	0	8	10	13	1	9.
Diseases of the musculoskeletal system	1	0	0	0	0	1	11.
Diseases of the genitourinary system	7	1	3	3	0	0	12.
Ill-defined	13	0	1	3	8	1	16.
Missing[e]	18						
External causes	85	1	34	28	16	6	17.
Accidents	51	1	16	16	14	4	17.1
Suicide	33	0	17	12	2	2	17.2

Note: Subgroups of causes of death are marked in cursive, they are also included in main groups as defined by the EU shortlist.

Abbreviations: TC, testicular cancer; y, year.

[a] Eurostat. European Shortlist of Causes of Death, May 2012. Available from: http://ec.europa.eu/eurostat/ramon/nomenclatures/index.cfm?TargetUrl=LST_NOM_DTL&StrNom=COD_2012&StrLanguageCode=EN&IntPcKey=&StrLayoutCode=HIERARCHIC.

[b] Other malignant neoplasms of lymphoid and haematopoietic tissue
[c] Of which AIDS n=3
[d] Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism
[e] Missing death diagnosis was no longer given an R-diagnosis from 1996 and onwards