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Prognostic factors for relapse in patients with clinical stage I testicular non-seminoma: A nationwide, population-based cohort study

Thomas Wagner [a,b,*], Birgitte Grønkær Toft [b], Jakob Lauritsen [a], Mikkel Bandak [a], Ib Jarle Christensen [c], Birte Engvad [d], Michael Kreiberg [a], Mads Agerbæk [e], Lars Dysager [f], Andreas Carus [g], Josephine Julie Rosenvilde [a], Daniel Berney [h,1], Gedske Daugaard [a,1]

[a] *Department of Oncology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark*
[b] *Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark*
[c] *Department of Pathology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Borgmester Ib Juuls vej 1, 2730 Herlev, Denmark*
[d] *Department of Pathology, Odense University Hospital, J. B. Winsløws vej 15, Winsløwsparken 15, 5000 Odense C, Denmark*
[e] *Department of Oncology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark*
[f] *Department of Oncology, Odense University Hospital, J. B. Winsløws vej 15, Winsløwsparken 15, 5000 Odense C, Denmark*
[g] *Department of Oncology, Aalborg University Hospital, Hobrovej 18–22, 9000 Aalborg, Denmark*
[h] *Centre of Cancer Biomarkers and Biotherapeutics, Barts Cancer Institute, Charterhouse Square, Queen Mary University of London, London, UK*

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ABSTRACT

Background: Approximately 30% of patients with clinical stage I non-seminoma (CSI-NS) relapse. Current risk stratification is based on lymphovascular invasion (LVI) alone. The extent to which additional tumor characteristics can improve risk prediction remains unclear.
Objective: To determine the most important prognostic factors for relapse in CSI-NS patients.
Design, setting, and participants: Population-based cohort study including all patients with CSI-NS diagnosed in Denmark between 2013 and 2018 with follow-up until 2022. Patients were identified in the prospective Danish Testicular Cancer database. By linkage to the Danish National Pathology Registry, histological slides from the orchiectomy specimens were retrieved.
Outcome measurements and statistical analysis: Histological slides were reviewed blinded to the clinical outcome. Clinical data were obtained from medical records. The association between prespecified potential prognostic factors and relapse was assessed using Cox regression analysis. Model performance was evaluated by discrimination (Harrell's C-index) and calibration.
Results: Of 453 patients included, 139 patients (30.6%) relapsed during a median follow-up of 6.3 years. Tumor invasion into the hilar soft tissue of the testicular hilum, tumor size, LVI and embryonal carcinoma were independent predictors of relapse. The estimated 5-year risk of relapse ranged from < 5% to > 85%, depending on the number of risk factors. After internal model validation, the model had an overall concordance statistic of 0.75. Model calibration was excellent.
Conclusion and relevance: The identified prognostic factors provide a much more accurate risk stratification than current clinical practice, potentially aiding clinical decision-making.

1. Introduction

More than 70% of patients with testicular germ cell cancer (TGCC) are diagnosed with clinical stage I (CSI) disease, of whom approximately one-third have non-seminoma (NS). [1] After orchiectomy alone, nearly 30% will relapse within five years of follow-up. [2,3] Post-orchiectomy options include surveillance, with treatment reserved for patients who relapse, or risk-adapted treatment with surveillance for those at low risk of relapse and adjuvant treatment for those at high risk of relapse. [4,5] Risk stratification and therapeutic decision-making have been based on lymphovascular invasion (LVI) for decades. [4,5] However, around 50% of patients with LVI will not relapse and are therefore exposed to

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unnecessary treatment. Similarly, approximately 15% of patients without LVI will relapse. [6] Thus, there is an unmet clinical need to define additional risk factors that can improve current prognostication, which could be achieved by integrating other tumor characteristics. Embryonal carcinoma (EC) has been associated with an increased risk of relapse, [2,3,6,7] but is not integrated into current risk stratification. Since LVI is most often caused by EC, [5,6] it has been controversial whether EC is an independent prognostic factor. [4,5] Moreover, it has been unclear whether it is only the presence of EC or the percentages of EC in the tumor that provide prognostic information. [6] The prognostic significance of other potential risk factors has been largely unexplored. [6,8,9] To our knowledge, Denmark is the only country in the world where all patients with CSI-NS have been managed with surveillance for decades and no adjuvant therapy administered, which offers a unique opportunity to assess risk factors for relapse in an unselected population with extended follow-up.

We therefore examined potential histopathological risk factors for relapse in a truly unselected population-based cohort assessed with a contemporary pathology review. The aims were to improve risk prediction and provide evidence-based risk estimates for relapse based on a prognostic model with identified risk factors.

2. Patients and methods

2.1. Study population and data sources

Detailed information about the study cohort and methods, including prognostic factors for relapse after orchiectomy for stage I seminoma, has been published previously. [10,11] In brief, all patients with CSI TGCC diagnosed in Denmark between 1 January 2013 and 31 December 2018 were identified in the prospective Danish Testicular Cancer (DaTeCa) database. [12] By individual-level data linkage to the Danish National Pathology Registry, [13] using the civil registration number, [14] the histologic slides from the orchiectomy specimens were collected and converted into digital images (using a Hamamatsu NanoZoomer XR scanner, Hamamatsu Photonics, Hamamatsu city, Japan). Information on clinical and follow-up data, vital status, emigration, and time of death was obtained by medical record review and by linkage to the Danish Civil Registration System. [14] Exclusion criteria were prior TGCC, synchronous TGCC, registration in the Register of Human Tissue Utilisation, [13] orchiectomy abroad, loss to follow-up within 30 days of orchiectomy and not CSI-NS disease.

This study is reported in accordance with STROBE and TRIPOD (Supplementary).

The study protocol was approved by the Regional Ethics Committee, the Danish Patient Safety Authority and the Data Protection Agency.

2.2. CSI disease and standard of care in Denmark

Patients had CSI disease if the post-orchiectomy serum tumor markers (STMs) α-fetoprotein (AFP) and β-human chorionic gonadotropin (β-hCG) were normalized and a CT-scan of the thorax, abdomen, and pelvis was without evidence of metastases. Patients with elevated STMs at orchiectomy had weekly measurements until normalization, confirming CSI disease. Following staging, all patients were offered a uniform 5-year surveillance program at three university hospitals, as previously described. [2,12,15] None received adjuvant treatment, regardless of adverse pathological features in the orchiectomy specimens (pT1–4).

2.3. Procedures

Three genitourinary pathologists (TW, BGT, and DB) were involved in the review process. Initial concordance sessions on testis pathology were held in person. In case of discordance between the review and initial reporting pathologist or other disagreement, digital images were reviewed by at least two pathologists, and consensus was reached. All histologic slides were reviewed with uniform reporting according to the International Collaboration on Cancer Reporting data set, [16] blinded to the clinical outcome. The following histopathological features were assessed: tumor necrosis, LVI, the percentages of each histological tumor types, pagetoid rete testis involvement, and invasion of either rete testis, hilar soft tissue, tunica albuginea, tunica vaginalis, epididymis or the spermatic cord, as prespecified in the study protocol, [10] and in Supplementary Table 1. The tumor diameter was recorded as stated in the original pathology report. In cases of multifocality, the tumor diameter was recorded as the largest diameter of the largest focus as recommended. [16] The percentages of the histological tumor types EC, yolk sac tumor (YST), teratoma, choriocarcinoma (CC) and seminoma were recorded in each case by eyeballing assessment of morphological features, according to the WHO 5th edition 2022 classification. [17].

Medical record review was performed by TW, JL, GD or MB, obtaining information on age at primary diagnosis (date of orchiectomy), pre- and post-orchiectomy levels of lactate dehydrogenase (LDH), AFP and β-hCG, radiological investigations, relapse status, and in case of relapse, additional data were collected. A nationwide access to medical records secured follow-up information on internal migrants. Relapse was defined as: a confirmed STM relapse (β-hCG, AFP) and/or radiological signs of relapse and/or histologically verified relapse, leading to subsequent treatment (chemotherapy and/or surgery). [4,10] The time point for relapse was defined as the date of biopsy or surgery in case of histologically proven relapse; if a relapse was defined by radiologic imaging and/or elevated STMs, the date when relapse was determined in the medical record was used. A joint decision was made in case of doubt about the clinical stage at primary diagnosis or relapse status. Follow-up information was updated in July 2022.

2.4. Statistical analyses

Relapse-free survival was defined as the time from orchiectomy until detection of the first relapse or TGCC death. Patients were censored at time of emigration (6), death due to other causes (5), metachronous TGCC (4), loss to follow-up (0), or end of study (July 2022), whichever came first. The Kaplan-Meier method was used to estimate the cumulative risk of relapse. The prespecified explanatory variables for relapse were analyzed using the Cox proportional hazards model, entering covariates as continuous, if applicable, or as categorical variables, primarily as binary. Model assumptions of proportional hazards and linearity were assessed by martingale residuals. For variables evaluated on a continuous scale (age, tumor size, tumor necrosis, AFP, LDH, β-hCG, and the percentages of the different histological tumor types), the functional form as well the proportionality assumption was assessed using martingale residuals and resampling. Those not fulfilling the criteria, were then scored as categorical variables, initially as binary. To evaluate whether a more detailed binning of the percentages of the different tumor types would improve the model fit, these were further categorized into absent vs present but not predominant (non-predominant) vs predominant, with predominant defined as the histologic subtype present in the greatest proportion. [18,19] A similar categorization was done with grouping into absent vs < 50% vs $\geq$ 50%. All variables were considered for inclusion in a multivariable Cox regression model irrespective of their univariable association with relapse. The full model was simplified with backward selection using a p-value of 0.05 as the exclusion criterion. The analyses were based on complete case data with increased sample size when removing a variable with missing values. This procedure included 10-fold cross-validation of the results, and interaction terms were evaluated. [20] The Cox model results were presented by hazard ratio (HR) estimates, with 95% Cis, test p-values, and graphical presentation. The predictive accuracy of the final model was assessed by examining discrimination and calibration measures. Discrimination was calculated with Harrel's concordance index (c-index). Additionally, a cumulative time-dependent receiver operating characteristic (ROC)

curve at five years after orchiectomy was plotted. Calibration was assessed with a calibration plot. P-values < 0.05 were considered significant. Database management and statistical calculations were performed using SAS (version 9.4), SPSS (version 28.0.1.0), and R (the RMS package). [21].

3. Results

In total, 1486 patients were registered in the DaTeCa database with CSI TGCC, of whom 453 were included with CSI-NS, Supplementary Figure 1. Table 1 shows the clinical characteristics and histopathological features on pathology review; additional characteristics are provided in Supplementary Table 2. During a median follow-up of 6.3 years (IQR 3.2–9.5), 139 patients (30.6%) relapsed; graphical presentation in Supplementary Figure 2. Kaplan-Meier estimate of time to relapse showed that 50% of observed relapses occurred within five months (0.41 years (IQR, 0.26–0.67)). Of the 139 incident relapses, 64 (46%) were confirmed by imaging in combination with elevated STMs, 46 (33%) were histologically confirmed by biopsy or lymphadenectomy,19 (14%) by elevated STMs alone, and 10 (7%) by imaging alone. In total, 132 relapsing patients (95%) belonged to the good prognostic group, while the remaining seven (5%) belonged to the intermediate group according to the International Germ Cell Cancer Collaborative Group (IGCCCG). [22] Treatment of primary relapsed disease consisted of chemotherapy (bleomycin, etoposide and cisplatin) in 135 patients (97.1%), and surgery alone in three patients (2.2%). One patient (0.7%) initially received radiotherapy (a misclassification of pure seminoma). In total, 29 patients underwent post-chemotherapy surgery. Of the total seven deaths (1.5%), two died of TGCC or treatment of TGCC after relapsing.

Tumor size, tumor necrosis, LVI, EC, teratoma, LDH, and invasion of tunica albuginea, rete testis, hilar soft tissue, epididymis, and spermatic cord were all associated with risk of relapse in univariable analysis (Table 2); graphical presentations are provided in Supplementary Figures 3–21. The final multivariable model consisted of LVI (present vs absent), hilar soft tissue invasion (present vs absent), tumor size (log2), and EC categorized into three groups (absent vs non-predominant vs predominant) (Table 3). The estimated 5-year risk of relapse based on the model is provided in Fig. 1a and b. In patients with small tumors and neither hilar soft tissue invasion, LVI or EC, the 5-year risk of relapse was < 5%. In contrast, in patients with large tumors harboring LVI, EC predominant histology and hilar soft tissue invasion, the 5-year risk of relapse was > 85%. Fig. 2 shows the observed cumulative risk of relapse among individuals stratified by the most common combinations of risk factors and with tumor size dichotomized by the median. The optimism-corrected predictive index (C-index) of the model was 0.75, and the 5-year ROC showed good discrimination and relapse prediction, Supplementary Figure 23. The calibration showed excellent agreement between the predicted 5-year risk of relapse and actual observations, Supplementary Figure 24. The novel model provided better discriminability than a Cox-provided model based on the current risk stratification using LVI status with respect to C-index (novel model, 0.75; previous model, 0.67). The estimated 5-year risk of relapse for patients with LVI was 59% (95% CI, 50 to 66; n = 152) compared to 17% (95% CI, 12 to 21; n = 301) in patients without LVI (graphical presentation in Supplementary Figure 3).

4. Discussion

In this large population-based cohort of patients with CSI-NS assessed with a contemporary pathology review, we identified tumor invasion into the hilar soft tissue of the testicular hilum and tumor size as novel predictors of relapse. We confirmed the prognostic significance of LVI and established that EC is a strong independent predictor of relapse. Different combinations of the identified risk factors markedly improved prognostication of the patients compared to current risk stratification. Thus, our prognostic model was able to identify patients at

Table 1

Baseline clinical characteristics and the histopathological features of the testicular tumors on pathology review of included patients with clinical stage I non-seminoma. Predominant histology was defined as the histologic subtype present in the greatest proportion (i.e. at a level greater than any other histological type).

Characteristics	Patient cohort (n = 453)
Demographics	
Median age, years (IQR, range)	31 (25-39,15-84)
Pre-orchiectomy serum tumor markers	
β-hCG, No. (%)	
Normal (< 2 IU/L)[a]	201 (44)
Elevated	252 (56)
Median in elevated cases, IU/L (IQR, range)	28 (8-167, 2-30000)
AFP, No. (%)	
Normal (< 12 IU/L)[b]	248 (55)
Elevated	205 (45)
Median in elevated cases, IU/L (IQR, range)	111 (36-326, 12-10260)
LDH, No. (%)	
Normal (≤ ULN)[c]	282 (62)
Elevated	163 (36)
Unknown	8 (2)
Median in elevated cases, U/L (IQR, range)	251 (220-284, 206-1421)
Histopathological features	
Tumor laterality, No. (%)	
Right	229 (51)
Left	224 (49)
Median tumor size, cm (IQR, range)	3.2 (2.1-4.5, 0.1-16)
Multifocal tumor, No. (%)	
Absent	372 (82)
Present	81 (18)
Tumor necrosis, No. (%)	
Absent	95 (21)
Present	358 (79)
Median in cases with tumor necrosis present, percentages (IQR, range)	10 (5-20, 1-98)
EC, No. (%)	
Absent	76 (17)
Present	377 (83)
Non-predominant histological type	172 (38)
Predominant histological type	205 (45)
Median in cases with embryonal carcinoma (EC), percentages (IQR, range)	49 (20-80, 1-100)
Teratoma, No. (%)	
Absent	130 (29)
Present	323 (71)
Non-predominant histological type	191 (42)
Predominant histological type	132 (29)
Median in cases with teratoma, percentages (IQR, range)	30 (10-55, 1-100)
YST, No. (%)	
Absent	149 (33)
Present	304 (67)
Non-predominant histological type	276 (61)
Predominant histological type	28 (6)
Median in cases with yolk sac tumor, percentages (IQR, range)	10 (5-20, 1-100)
Seminoma component, No. (%)	
Absent	199 (44)
Present	254 (56)
Non-predominant histological type	166 (37)
Predominant histological type	88 (19)
Median in cases with a seminoma component, percentages (IQR, range)	25 (5-60, 1-99)
CC, No. (%)	
Absent	375 (83)
Present	78 (17)
Non-predominant histological type	78 (17)
Predominant histological type	0
Median in cases with choriocarcinoma, percentages (IQR, range)	5 (2-10, 1-30)
LVI, No. (%)	
Absent	301 (66)
Present	152 (34)
Pagetoid rete testis involvement, No. (%)	

(continued on next page)

Table 1 (*continued*)

Characteristics	Patient cohort (n = 453)
Absent	285 (63)
Present	159 (35)
Unknown	9 (2)
Rete testis invasion, No. (%)	
Absent	295 (65)
Present	150 (33)
Unknown	8 (2)
Hilar soft tissue invasion, No. (%)	
Absent	375 (83)
Present	78 (17)
Epididymis invasion, No. (%)	
Absent	438 (96.5)
Present	14 (3)
Unknown	1 (0.5)
Spermatic cord invasion, No. (%)	
Absent	448 (99)
Present	5 (1)
Tunica albuginea invasion, No. (%)	
Absent	314 (69)
Present	139 (31)
Tunica vaginalis invasion, No. (%)	
Absent	451 (99.5)
Present	2 (0.5)

Abbreviations: AFP, alpha-fetoprotein; β-hCG, β-human chorionic gonadotropin; LDH, lactate dehydrogenase; LVI, lymphovascular invasion; EC, embryonal carcinoma; YST, yolk sac tumor; CC, choriocarcinoma

[a] In one of the treating institutions (No. of included patients = 81), normal reference levels of β-hCG were < 5 IU/L. However, the absolute pre-orchiectomy value of β-hCG was registered in each case on every institution upon medical record review.

[b] In two of the treating institutions (No. of included patients = 250), normal reference levels of AFP were < 7 IU/L. However, the absolute pre-orchiectomy value of AFP was registered in each case on every institution upon medical record review

[c] ULN, Upper limit of normal (= 205 U/L, except in three cases)

very low risk for relapse (5-year rate of <5%), and very high risk for relapse (5-year rate of >85%).

Disease-specific survival for patients with CSI-NS approaches 100% regardless of the post-orchiectomy treatment strategy. [3] Adjuvant therapy is unnecessary and harmful for about 70% of patients, who are cured by orchiectomy alone. This underscores the importance of identifying high-risk patients more likely to benefit from adjuvant treatment. Current guidelines indicate that adjuvant therapy should be considered for patients with LVI. However, as shown in a recent meta-analysis, [6] and validated in our study, prognostication and therapeutic decision-making based on LVI alone is too simplistic, resulting in overtreatment of around 50% of the patients cured by orchiectomy alone. Furthermore, our findings show that patients with LVI harbour a 15% to 85% risk of relapse depending on the presence of additional risk factors. The identified risk factors in the present study provide essential information in the post-orchiectomy counselling of CSI-NS patients with the potential for improvement of individualized post-orchiectomy management.

The present study confirms that non-risk-adapted surveillance remains a viable approach. However, the identified prognostic factors and their combinations provide a much more accurate risk assessment than previously. Adjuvant therapy for non-seminoma patients typically involves a single cycle of BEP. [4,5] The updated SWENOTECA study and the 111 Study suggest a potential reduction in the risk of relapse to approximately 3%. [23,24] These studies included 258 and 236 high-risk patients, with median follow-up times of 7.9 years and 4.1 years, respectively. Except for one patient in the IGCCCG intermediate group, all others were classified into the good prognostic group. These findings must, however, be interpreted cautiously, as the single high-risk criterion was the presence of LVI. Consequently, the efficacy of adjuvant treatment in a true high-risk group remains unclear based on current

Table 2

Results of the univariable Cox regression analysis for time to relapse.

Characteristic	HR (95% CI)	p-value
Age, years	1.00 (0.99-1.02)	0.9310
AFP (log2)	1.00 (0.94-1.05)	0.9130
LDH (log2)	1.90 (1.38-2.62)	< 0.0001
β-hCG (log2)	1.01 (0.96-1.06)	0.7626
Tumor size (log2)	1.32 (1.06-1.63)	0.0122
Tumor multifocality (present vs absent)	0.75 (0.47-1.21)	0.2433
Tumor necrosis (present vs absent)	4.70 (2.39-9.24)	< 0.0001
Pagetoid rete testis involvement (present vs absent)	1.10 (0.78-1.55)	0.5861
Rete testis invasion (present vs absent)	2.43 (1.74-3.39)	< 0.0001
Hilar soft tissue invasion (present vs absent)	2.71 (1.89-3.89)	< 0.0001
LVI (present vs absent)	4.87 (3.44-6.88)	< 0.0001
Epididymis invasion (present vs absent)	4.19 (2-20-8.00)	< 0.0001
Spermatic cord invasion (present vs absent)	6.56 (2.41-17.82)	0.0002
Tunica albuginea invasion (present vs absent)	1.92 (1.37-2.69)	0.0001
Tunica vaginalis invasion (present vs absent)	2.32 (0.33-16.63)	0.4006
EC, dichotomized (present vs absent)	3.54 (1.80-6.97)	0.0002
EC, categorized		
Absent	1 [Reference]	
Non-predominant	2.20 (1.07-4.53)	0.0319
Predominant	4.94 (2.49-9.81)	< 0.0001
Teratoma, dichotomized (present vs absent)	0.46 (0.33–0.65)	< 0.0001
Teratoma, categorized		
Absent	1 [Reference]	–
Non-predominant	0.59 (0.41-0.85)	0.0047
Predominant	0.29 (0.18-0.48)	< 0.0001
Seminoma, dichotomized (present vs absent)	1.31 (0.93-1.85)	0.1211
Seminoma, categorized		
Absent	1 [Reference]	–
Non-predominant	1.62 (1.13-2.32)	0.0095
Predominant	0.83 (0.50-1.37)	0.499
YST, dichotomized (present vs absent)	0.95 (0.67-134)	0.7545
YST, categorized		
Absent	1 [Reference]	–
Non-predominant	0.97 (0.68-1.39)	0.8636
Predominant	0.74 (0.33-1.27)	0.4488
CC, dichotomized[d] (present vs absent)	0.68 (0.41-1.12)	0.1270

Abbreviations: AFP, alpha-fetoprotein; β-hCG, β-human chorionic gonadotropin; LDH, lactate dehydrogenase; LVI, lymphovascular invasion; EC, embryonal carcinoma; YST, yolk sac tumor; CC, choriocarcinoma

[d] No tumor had a predominant CC component

data. There is limited data on patients treated with adjuvant therapy who relapse. An international retrospective analysis, albeit small in scale, reported a worse prognosis for patients relapsing after adjuvant BEP compared to those with de novo metastatic disease, causing concerns about delayed onset relapse and cisplatin chemoresistance unable to be salvaged. [25] Furthermore, there is limited available data with very long-term follow-up relevant for assessing the risk of treatment-related morbidity, particularly in terms of cardiovascular diseases and second malignancies. Further research is necessary to provide insights into the optimal treatment for the high-risk group defined in the current study. Another viable post-orchiectomy treatment option is primary retroperitoneal lymph node dissection (RPLND). [4,5, 26] However, existing studies do not provide clarity on the criteria used to select patients for RPLND, and there is a lack of randomized studies comparing RPLND to surveillance with clearly defined entry criteria.

Table 3

Results of the final multivariable Cox model for time to relapse.

Characteristic	HR, (95% CI)	p-value
LVI (present vs absent)	3.48 (2.38-5.10)	< 0.0001
EC[e]		
Absent	1 [Reference]	–
Non-predominant	2.49 (1.20-5.15)	0.0138
Predominant	4.06 (1.98-8.32)	0.0001
Hilar soft tissue invasion (present vs absent)	1.70 (1.17-2.48)	0.0056
Tumor size (log2)	1.60 (1.25-2.03)	0.0001

Abbreviations: HR, hazard ratio; LVI, lymphovascular invasion; EC, embryonal carcinoma; YST, yolk sac tumor.

[e] The predominant categorization (absent vs non-predominant vs predominant) and categorization based on the cut-off value of 50% (absent vs < 50% vs ≥ 50%) showed similar results. The predominant categorization was chosen as we believe it is less prone to intra- and interobserver variability and more easily incorporated into clinical practice. It is simpler, easier, and more reproducible to determine whether a tumor subtype is the predominant component rather than estimating if it constitutes more or less than 50%. In complex mixed tumors with several components (e.g. a tumor composed of 40% EC, 30% YST, 20% seminoma and 10% teratoma), the EC component may still predominate even though the component is not larger than an arbitrary 50% cut-off point).

Examining relapses in the high-risk group defined in the present study, reveals that 38% of patients experienced relapse outside the retroperitoneum, indicating that RPLND would not have been beneficial. This underscores the need for additional studies to clarify which patient group could benefit from upfront operative intervention. RPLND can be an alternative to chemotherapy for patients in relapsed stage IIA/IIB disease. [26,27]

The testicular hilum, which consists of rete testis and hilar soft tissue (illustrated in Supplementary Figure 25), is the predominant pathway of extra-testicular tumor spread, [28,29] and an established predictor of relapse in CSI seminoma. [11] Yet, its prognostic significance in CSI-NS has been poorly investigated. To our knowledge, only our research group has investigated rete testis invasion as a potential risk factor and

found it prognostic for relapse but lacked data on hilar soft tissue invasion. [2] The present study found rete testis invasion highly significant in univariable analysis, but the prognostic significance was superseded by hilar soft tissue invasion in the multivariable analysis, reflecting the more advanced tumor spread. Tumor size has been extensively studied in patients with CSI seminoma and proven prognostic for relapse in several studies. [30] Still, there is a lack of data on CSI-NS, with only six small studies investigating its prognostic significance. [31–36] Roeleveld et al. [32] found tumor size significantly associated with relapse, while the other studies found no association. The findings from our large cohort indicate that these studies were statistically underpowered, and tumor size should be considered an important risk factor for relapse. In contrast to seminomas, where accurate assessment of tumor size is often challenged by intertubular growth and multifocality, [11,37] estimation of tumor size in NS is usually more straightforward. The present study confirms the prognostic significance of LVI, and that EC is the primary angio-invasive histologic tumor type. However, we established that EC is a strong independent predictor of relapse and should be used as an additional prognosticator. Furthermore, including a simple-to-use three-tier categorization of EC markedly improved risk stratification compared to dichotomizing EC.

Unlike most other cancer types, there is currently no validated molecular biomarker for assessing the risk of relapse in patients with CSI-NS disease, and current risk assessment, therefore, relies solely on the histological features in the testicular tumors. Although novel microRNAs (miRNAs) hold promises in the future management of TGCC with higher sensitivity and specificity in detecting relapse compared to the conventional STMs, [38] a recent study revealed that miRNA371a-3p (M371 test) levels are not predictive of relapse. [39] However, ongoing research aims to clarify whether miRNA can significantly complement the provided prognostic model. Furthermore, recent research has indicated an association between the loss of expression of the pluripotency regulatory factor, DAZL, and an increased risk of relapse. [40] Collaborations are underway to validate these findings.

This study has limitations. Despite almost no missing values of the explanatory variables, we cannot exclude inconsistent sampling at macroscopic examination. However, national guidelines on handling
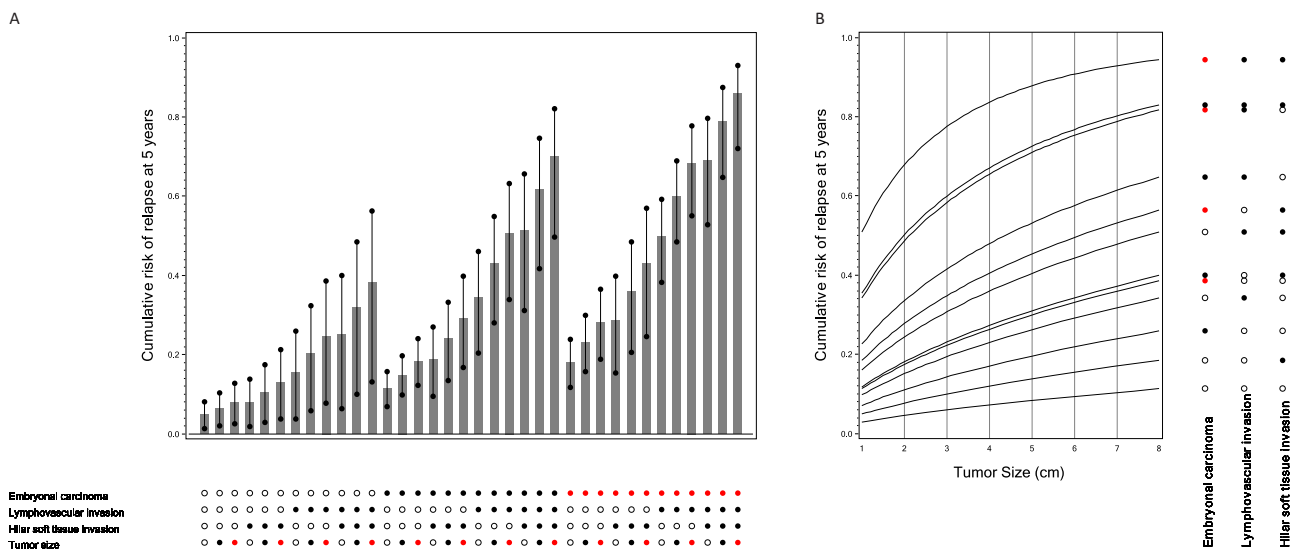

Fig. 1. Estimated 5-year cumulative risk of relapse of the risk factors included in the final multivariable model. Circles illustrate a single risk factor, and the presence of possible combinations of different risk factors are marked with solid circles. For the Embryonal carcinoma (EC) variable, a circle without a mark represents absence of EC in the tumor, a black mark represents non-predominant EC, and a red mark represents EC predominant histology. (A) Estimates with 95 CI and fixed tumor sizes. With no established cut-off values of tumor size, we chose to include tumor sizes based on the datasets median and quartiles. Thus, a circle without a mark represents the 25th percentile (2.1 cm), a black mark represents patients with tumor size at the median (3.2 cm), and a red mark represents the 75th percentile (4.5 cm). For patients with a small tumor (2.1 cm) and without any other risk factor, the estimated 5-year risk of relapse is 5% (95% CI, 1 to 8). For patients with a large tumor (4.5 cm) in combination with predominant EC histology, lymphovascular invasion (LVI), and hilar soft tissue invasion, the estimated 5-year risk of relapse is 86% (95% CI, 72 to 93). (B) Estimates for the different combinations of LVI, EC, and hilar soft tissue invasion as a function of tumor size.
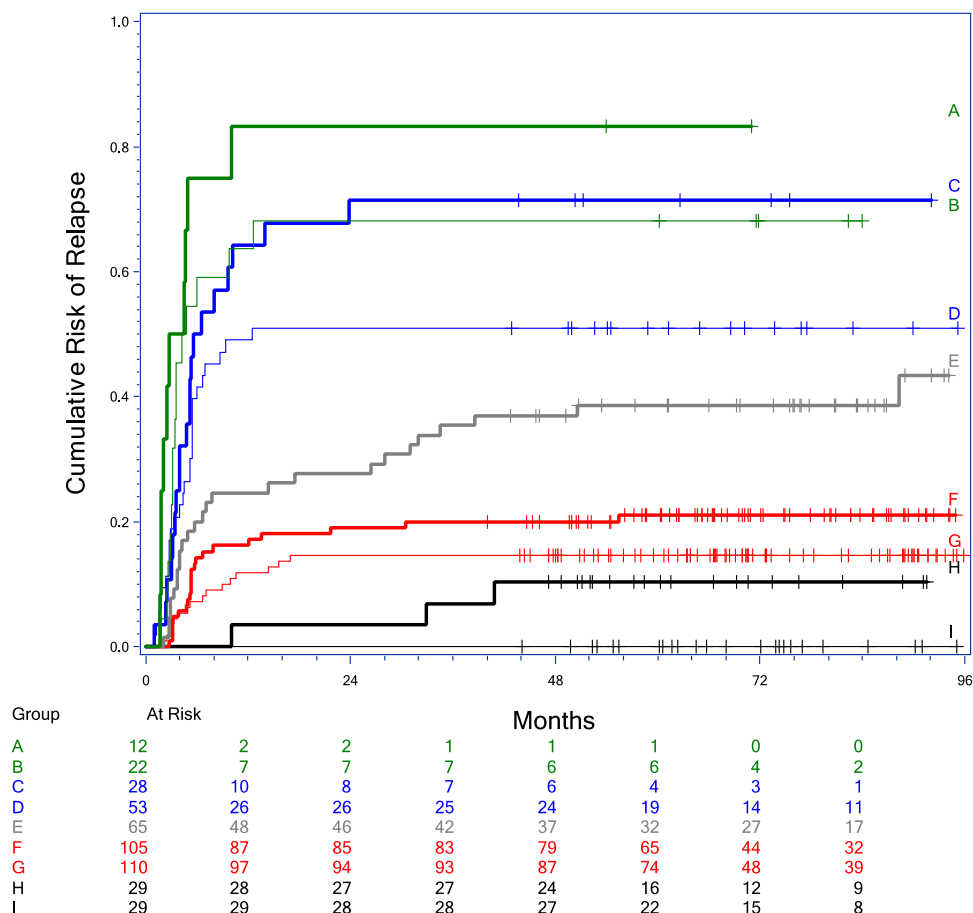
Fig. 2. 5-year cumulative risk of relapse stratified by different risk groups. The most common combinations of risk factors are illustrated separately. The remaining combinations are grouped into "Other" in Group E (n = 65, complete data including different combinations are provided in Supplementary Figure 22). For the Embryonal carcinoma (EC) variable, EC- represents absence of EC in the tumor, EC+ represents non-predominant EC, and EC+ + represents predominant EC histology. LVI- and LVI+ , hilar soft tissue invasion- and hilar soft tissue invasion+ represents absence and presence of lymphovascular invasion (LVI) and hilar soft tissue invasion, respectively. Tumor size is stratified by the median (3.2 cm). Group A: LVI+ , EC+ +, hilar soft tissue invasion+ , and tumor size > median. Group B: LVI+ , EC+ +, hilar soft tissue invasion+ , and tumor size ≤ median. Group C: LVI+ , EC+ +, hilar soft tissue invasion-, and tumor size > median. Group D: LVI+ , EC+ +, hilar soft tissue invasion-, and tumor size ≤ median. Group E: "Other". Group F: EC+ + or EC+ , LVI-, hilar soft tissue invasion-, and tumor size > median. Group G: EC+ + or EC+ , LVI-, hilar soft tissue invasion-, and tumor size ≤ median. Group H: EC-, LVI-, hilar soft tissue invasion-, and tumor size > median. Group I: EC-, LVI-, hilar soft tissue invasion-, and tumor size ≤ median.

and sampling of orchiectomy specimens have been standardized in Denmark for many years, [15] and per international recommendations. [16] Although we applied internal validation to estimate optimism-corrected performance, external validation is the gold standard for assessing the generalizability of prediction models. [20] Identifying a similar cohort outside of Denmark seems extremely challenging since adjuvant chemotherapy has been implemented for "high-risk" patients in most other countries. This makes unselected cohorts of patients followed on a surveillance-only program rare. However, the biologically plausible associations of the identified risk factors for relapse strongly suggest that these findings are highly valid.

5. Conclusion

Tumor invasion into the hilar soft tissue of the testicular hilum, tumor size, LVI and EC were independent predictors of relapse in patients with CSI-NS. Different combinations of the identified risk factors markedly improved prognostication compared to current risk stratification. The provided prognostic factors are easily incorporated into routine clinical practice and enable providers and patients to make more informed decisions about post-orchiectomy management. The present data provide a new foundation for future research investigating risk-adapted follow-up and treatment strategies.

Contributors

TW, GD, BGT, and DB developed the conception and design of the study, obtained permissions and funding. MB and JL were involved in the study design. IJC was mainly responsible for statistical design and analysis. TW, BGT, and DB did the pathology review. TW, GD, JL, and MB did the medical record review. MA, AC and LD did re-review of medical records in case of uncertainties (few cases). TW drafted the manuscript. JL, MB, DB, BGT, IJC, MK, JJR, AC, MA, LD, BE, and GD contributed to analysis and interpretation of the results and feedback. All authors participated in the critical revision and approval of the manuscript. TW, IJC and JL had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. TW had the final responsibility for the decision to submit for publication.

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CRediT authorship contribution statement

Lars Dysager: Writing – review & editing, Methodology. **Andreas Carus:** Writing – review & editing, Methodology. **Michael Kreiberg:** Writing – review & editing, Methodology. **Mads Agerbæk:** Writing – review & editing, Methodology. **Ib Jarle Christensen:** Software, Methodology, Formal analysis. **Birte Engvad:** Writing – review & editing, Methodology. **Mikkel Bandak:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Birgitte Grønkær Toft:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Gedske Daugaard:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Jakob Lauritsen:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Formal analysis. **Josephine Julie Rosenvilde:** Writing – review & editing, Methodology. **Thomas Wagner:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Daniel Berney:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

Dan Berney is supported by Orchid. Gedske Daugaard declares honoraria from Bayer, Astellas, Janssen, MSD, Pfizer and AAA. Jakob Lauritsen declares honoraria from MDS. All other authors declare no competing interests.

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Data sharing statement

Due to data protection laws, researchers need to apply the Danish Health Data Authority to have access to the underlying person-level data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.114025.

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