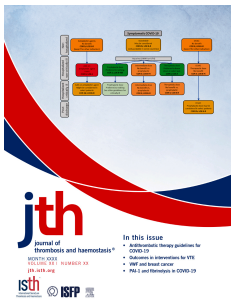

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PII: S1538-7836(25)00070-4

DOI: https://doi.org/10.1016/j.jtha.2025.01.022

Reference: JTHA 953

To appear in: *Journal of Thrombosis and Haemostasis*

Received Date: 25 July 2024

Revised Date: 25 December 2024

Accepted Date: 27 January 2025

Please cite this article as: Moik F, Terbuch A, Sprakel A, Pichler G, Barth DA, Pichler R, Rainer P, Silbernagel G, Mannweiler S, Jost PJ, Ahyai SA, Bauernhofer T, Hutterer GC, Pichler M, Arterial thromboembolic events in testicular cancer patients: Short- and long-term incidence, risk factors and impact on mortality, *Journal of Thrombosis and Haemostasis* (2025), doi: https://doi.org/10.1016/j.jtha.2025.01.022.

Arterial thromboembolic events in testicular cancer patients: Short- and long-term incidence, risk factors and impact on mortality

Short Title:
Arterial thromboembolic events in testicular cancer

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Word count
Abstract: 244 words
Word count: 3,656 words
Tables: 3
Figures: 4 Figures
Supplemental Appendix 1 (5 Suppl. Tables, 1 Suppl. Figure)

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Abstract

Background: Patients with testicular germ cell tumors (TGCT) have a high cancer-specific survival rate. We aimed to determine the short- and long-term risk of arterial thromboembolic events (ATE), their impact on mortality, and risk factors for ATE in TGCT patients.

Methods: Patients with TGCT treated between 1994-2020 were included in a single-center retrospective cohort study. The primary outcome was ATE (i.e., acute coronary syndrome, ischemic stroke, acute peripheral arterial occlusion). Cumulative incidences were obtained in competing risk analysis. The impact of ATE on mortality was analyzed in a multi-state model. Cox-regression was used to explore short-and long term ATE-risk factors.

Results: Overall, 1,277 patients were included (median age: 35 years; seminoma: 56%, 44% cisplatin-based chemotherapy). Cumulative ATE-incidences at 1-, 10-, and 25-years were 0.6% (95% confidence interval [CI]: 0.3-1.1), 2.6% (1.8-3.7), and 12.0% (8.7-15.9). ATE diagnosis was independently associated with increased all-cause mortality (age-adjusted transition hazard ratio: 4.61 [95%CI: 2.40-8.85], p<0.001). Cisplatin-based chemotherapy was associated with ATE-risk within 1 year after TGCT diagnosis (1.4% vs 0%, p<0.001), whereas no differences were observed thereafter. Regarding long-term ATE-risk, a point-based risk score was derived (age ≥35, smoking, LDH ≥250IU/L), which efficiently stratified ATE risk (Harrel´s C: 0.71 [95% CI: 0.63–0.78]), with cumulative ATE-incidences in low-, intermediate- and high-risk patients of 3.9%, 11.4%, and 22.7%, respectively.

Conclusions: ATE represent a common complication in TGCT survivors and are associated with increased mortality. A simple point-based score efficiently stratifies long-term ATE-risk, whereas cisplatin-based chemotherapy increased short-term ATE risk.

Key words

Testicular cancer, arterial thromboembolic events, testicular germ cell tumors, cardiovascular risk, cisplatin

Abbreviations

ACS: Acute coronary syndrome

AFP: alpha-fetoprotein

ATE: Arterial thromboembolic events

β-HCG: β human chorionic gonadotropin

BMI: body mass index

CS: clinical stage

HR: hazard ratio

IQR: interquartile ranges

LDH: lactate dehydrogenase

OS: Overall survival

TGCT: testicular germ-cell tumors

Introduction

Patients with testicular germ-cell tumors (TGCT) have a high cancer-specific survival, yet cardiovascular events represent an important secondary contributor to morbidity and mortality in certain patient subgroups, including those receiving platinum-based chemotherapy.[1-5] Especially, given high cure rates and a commonly young age at the time of diagnosis, the impact on short- and long-term mortality and morbidity of cardiovascular adverse events represent an important aspect during the treatment and follow-up care in TGCT patients.[6]

Arterial thromboembolic events (ATE), comprising acute coronary syndrome (ACS), ischemic stroke, and acute peripheral arterial occlusion, represent the most severe and life-threatening manifestations of underlying cardiovascular disease. In patients with TGCT, increased rates of cardiovascular complications have been reported, both during active treatment, as well as during long-term follow-up.[1,7] Regarding short-term cardiovascular risk, an increased risk of arterial- and venous thromboembolic events was previously reported specifically in high-risk subgroups of patients receiving platinum-based chemotherapy and those with more advanced cancer stages.[5,8,9] Beyond the initial treatment timeframe, an increased risk of cardiovascular disease has been reported in subsets of patients with TGCT, especially those receiving platinum-based chemotherapy.[1-5] Several risk factors for the development of future cardiovascular diseases in TGCT survivors have been reported in the past, including underlying cardiovascular comorbidities, treatment specific factors (especially cisplatin-based chemotherapy and radiotherapy), and modifiable patient-specific risk factors (e.g. smoking).[2,10]

However, in general, specific data on short-and long-term ATE rates are sparse and a refined understanding of ATE risk profiles and the underlying risk factors to identify high-risk sub-populations represents an urgent and unmet medical need. Importantly, ATE represent a potentially preventable medical condition, which might therefore enable the development of risk stratification models to be used as selection tools for primary prophylactic interventions that might ultimately lead to improved patient care.

Therefore, the aim of our present study was to determine detailed ATE risk profiles in a large and unselected cohort of patients with TGCT over a long-term follow-up period, to determine the potential impact of ATE on mortality, and to explore putative risk factors for their predictive utility towards short- and long term ATE risk.

Methods

Study design and patient cohort

In the present single-center cohort study, patients with histologically confirmed TGCT, diagnosed at a tertiary academic care center (Medical University Graz) between 01.01.1994-31.12.2020, were included. All data were retrospectively collected individually from electronic medical files of the patients and include baseline clinical co-variables, including patient demographics, comorbidities, cancer types and treatments, as well as data collected during the routine clinical follow-up of patients. The presence of medical comorbidities at study inclusion were defined as assigned by the treating physicians. Follow-up data availability included in-hospital medical records, as well as data from other federal state hospitals included in the Styrian Hospital Corporation (KAGES). Data collection and analyses were approved by the local institutional review board (Ethics Committee of the Medical University of Graz, Austria; document number No: 32-200 ex 19/20). Given the study's retrospective design, utilizing data from pre-existing electronic medical records, the requirement for written informed consent was waived by the local institutional review board. The study was conducted according to the relevant local and national guidelines and regulations including the Declaration of Helsinki.

Outcome definition

Patients were followed from the day of TGCT diagnosis. The primary outcome event of interest was defined as objectively confirmed ATE, comprising ACS, ischemic stroke, and acute peripheral arterial occlusion. Diagnosis of ATE had to be verified by objective diagnostic test. Detailed outcome definitions and objective measures for diagnosis are provided in **Supplemental Table 1**. All-cause mortality was considered as secondary outcome event of interest. The follow-up period was terminated at the day of an observed outcome event or censored at the day of the last contact with the patient without the occurrence of an outcome event.

Statistical analysis

Standard descriptive statistics were used to summarize patient-, cancer-, and treatment-specific baseline variables, including median and interquartile ranges (IQR) for continuous data, and absolute frequencies and percentages for count data, respectively. The reverse Kaplan-Meier method was applied to obtain median follow-up times. The absolute risk of ATE was obtained by estimating competing risk cumulative incidences over time, accounting for all-cause mortality as competing outcome event, to avoid an overestimation of absolute risks, utilizing

Gray´s test for between-group comparisons of cumulative incidence functions.[11] Overall survival (OS) estimates were obtained in conventional time-to-event analysis (Kaplan-Meier method). The association of ATE during follow-up on the risk of mortality was analyzed with ATE as a time dependent covariable in a Cox regression model, representing a unidirectional multi-state model, calculating the transition hazard ratio (tHR) quantifying the risk of death after ATE compared to the baseline risk before ATE diagnosis and to those without ATE during follow-up.[12] For better visualization, a landmark analysis was used, comparing OS estimates of patients according to the occurrence of ATE within the first 10 years of follow-up. Risk factors for ATE were explored in univariable- and multivariable Cox regression models, calculating HRs for ATE of candidate predictors during study follow-up. Moreover, a pragmatic point-based risk score was derived, including variables with a significant association in univariable analysis in the model derivation, with a subsequent cut-off value for omitting variables upon backwards selection of α=0.157 to identify the optimal model according to the Akaike information criterion (AIC).[13] The discriminatory performance of the score was quantified by obtaining the concordance-index Harrel´s C, with internal validation of the model via bootstrapping of 100 random samples. The proportional hazards assumption was tested graphically by plotting the cumulative hazard function for the Cox–Snell residuals versus the residuals themselves, and by hypothesis-testing whether the log hazard-ratio function is constant over time via Schoenfeld residuals (Supplemental Figure 1). All statistical analyses were performed with the commercially available package STATA 15.1 (Stata Corp., Houston, TX, USA).

Results

Study population

Overall, 1,277 patients were included in the present analysis. Patients´ median age at the time of cancer diagnosis was 35 years (IQR: 29-43). Seminoma was the most common histologic subtype (n=714, 56%), whereas 563 (44%) patients presented with non-seminoma subtypes (including at least one non-seminoma component). Further, most patients presented with early-stage cancer (clinical stage [CS] I, n= 928, 73%), while 349 (27%) patients had more advanced cancer stages (CS ≥II).

Common cardiovascular risk factors and comorbidities included a positive history of smoking in 488 (46% of patients with available data) patients, arterial hypertension in 95 (28%) patients, and diabetes mellitus in 28 (2%) patients. The median body mass index (BMI) was 24.9kg/m^2 (IQR: 22.9-27.5), with 142 (12%) patients at a BMI ≥30kg/m^2. Regarding anti-cancer treatment,

cisplatin-based chemotherapy was applied in 557 (44%) patients, with a median of 3 cycles (IQR:2-4). The median time from cancer diagnosis to initiation of chemotherapy was 23 days (IQR: 14-33). Most patients (98.3%) received their first cycle of chemotherapy within 3 months after cancer diagnosis. Further, radiotherapy was applied in 118 (9%) patients. Overall, 129 patients (10.7%) suffered a relapse during follow-up. Details on the baseline characteristics of the study cohort are displayed in **Table 1**. Detailed treatment-related data including initial and subsequent treatment regimens are provided in **Supplemental Table 2-3**.

Long-term risk of ATE

Over a median follow-up of 13.1 years (IQR: 6.5-19.4), we observed 64 cases of ATE. The most common type of ATE was ACS (n=38, 59%), followed by ischemic stroke (n=13, 20%), and acute peripheral arterial occlusion (n=13, 20%). The cumulative incidence of ATE during follow-up was 0.6% [95%CI: 0.3-1.1] at 1 year, 1.4% (95%CI: 0.9-2.3) at 5 years, 2.6% (95%CI: 1.8-3.7) at 10 years, 7.3% (95%CI: 5.5-9.6) at 20 years, and 12.0% (95%CI: 8.7-15.9) at 25 years. In **Figure 1**, the competing risk cumulative incidence function, accounting for all-cause mortality as competing outcome event, is visualized.

Impact on mortality

During the complete follow-up period, we observed 81 deaths, with corresponding 1-, 5-, 10-, 20-, and 25-year OS-estimates of 99.1% (95%CI: 98.4-99.5), 97.2% (95%CI: 96.0-98.0), 95.2% (95%CI: 93.7-96.3), 90.8% (95%CI: 88.4-92.8), and 88.5% (95%CI: 85.1-91.1), respectively. The occurrence of ATE during follow-up was associated with a substantially increased risk of all-cause mortality in a multi-state model, incorporating ATE as a time-dependent covariable in OS-analysis (tHR: 7.32 [95%CI: 3.93-13.64], $p<0.001$). This association prevailed upon multivariable adjustment for patient age (tHR: 4.61 [95%CI: 2.40-8.85], $p<0.001$). In patients who developed ATE, the estimated cumulative overall mortality at 1-, 5-, and 10-years after ATE was 5.0% (95%CI: 1.6-14.6), 8.6% (95%CI: 3.4-19.5), and 20.8% (95%CI: 10.9-37.6), respectively. In **Figure 2**, a landmark analysis is shown, displaying OS-estimates of patients according to the occurrence of ATE within the first 10 years after a TGCT diagnosis.

Overall, the adjudicated cause of death was determined to be testicular cancer-related in 27 patients (35% of deaths) and ATE-related in 13 (17%). Further, 59 patients (4.6%) were diagnosed with second primary malignancies at a median of 4.8 years after diagnosis of testicular cancer (IQR: 3.1-9.2). Of those, 8 patients died due to their second primary cancers.

Risk factors for ATE

In univariable analysis, a higher risk of ATE was observed for patients with higher age (HR per 10 years increase: 2.17 [95%CI: 1.74-2.71]; HR for age ≥35 years [i.e. median of overall distribution]: 3.60 [2.01-6.44], underlying diabetes mellitus (HR: 10.33 [95%CI: 4.35-24.52]), a positive history of smoking (HR: 1.98 [95%CI: 1.09-3.61]), and a BMI ≥30kg/m^2 (HR: 2.26 [95%CI: 1.09-4.70]). Regarding cancer specifics and its treatments, neither cancer stage nor histology were associated with ATE risk. Moreover, the application of cisplatin-based chemotherapy was not significantly associated with long-term ATE risk. In a time-dependent analysis, the HR for ATE after initiation of cisplatin-based chemotherapy was 1.19 (95%CI: 0.67-2.11). Patients who underwent radiotherapy had increased ATE rates (HR: 2.04 [95%CI: 1.13-3.71]). Further, baseline systemic levels of tumor markers were evaluated, with an observed increase in ATE risk for higher levels of lactate dehydrogenase (LDH), both on a continuous scale (HR per double of levels: 1.54 [95%CI: 1.09-2.17]), and upon dichotomization at the upper limit of the normal reference range (HR for LDH ≥250 IU/L: 2.97 [95%CI: 1.46-6.05]). This association prevailed upon multivariable adjustment for cancer stage and cycles of cisplatin-based chemotherapy (adjusted HR: 2.97 [95%CI: 1.17-7.56]). In contrast, levels of β human chorionic gonadotropin (β-HCG) and alpha-fetoprotein (AFP) were not associated with ATE risk. Details on the univariable associations of putative risk factors with prospective ATE risk are provided in **Table 2**. Similar risk patterns were observed upon analyzing the risk of ATE after 1 year, 5 years, and 10 years of follow-up (**Supplemental Table 4**).

Risk-score for ATE risk

Next, we evaluated variables demonstrating a statistically significant association with long-term ATE risk in univariable analysis (age ≥35, diabetes, smoking, BMI ≥30kg/m^2, radiotherapy, LDH ≥250 IU/L) in a multivariable model. Upon backwards selection, omitting variables in a stepwise process to a threshold of α=0.157 [8], an independent association with the risk of future ATE was observed for patients aged ≥35 years (HR 4.41 [95%CI: 2.00-9.72], positive smoking status (HR 2.02 [95%CI: 1.05-3.88]), and a baseline LDH level of ≥250 IU/L (HR: 3.43 [95%CI: 1.62-7.28]). Based on this model, a simplified point-based score was derived, assigning +1 point for patients ≥35 years of age at the time of diagnosis, a positive history of smoking, and baseline LDH values of ≥250 IU/L, respectively (**Supplemental Table 5**). An increasing risk of ATE was observed from patients with 0 points (n=211) to patients with 1 point (n=517) and ≥2 points (n=264), with corresponding cumulative ATE risks over a 25-year

follow-up period of 3.9% (95%CI: 0.6-12.7), 11.4% (95%CI: 4.6-21.6), and 22.7% (95%CI: 12.0-35.5), respectively. **Figure 3** displays cumulative incidence functions of the ATE-risk according to the calculated risk score. An internal validation via bootstrapping was performed to determine the performance abilities of the risk score, with an adjusted concordance index (Harrel's C) of 0.71 (95% CI: 0.63–0.78).

Risk factors for short-term ATE risk

Finally, we explored whether short-term risk factors for ATE (i.e., within 1 year after TGCT diagnosis) might differ from long-term ATE risk profiles. In univariable analysis, no increase in the 1-year ATE risk was observed for patients with higher age, diabetes, smoking, or a higher BMI.

Regarding cancer characteristics and therapies, the application of cisplatin-based chemotherapy was associated with 1-year ATE risk, with all nine ATE events observed during this timeframe occurring in patients treated with cisplatin-based therapy (cumulative 1-year incidence: 1.3% [95%CI: 0.5-2.5] *vs.* 0%, Gray´s test: p=0.003, **Figure 4**). Accordingly, a dose-dependent effect was observed as risk of ATE within one year after cancer diagnosis increased by the number of applied cisplatin-based chemotherapy cycles (HR per increase in chemotherapy-cycles: 1.38 [95%CI: 1.10-1.74]). Beyond 1 year of follow-up, no difference in ATE risk was observed according to the receipt of cisplatin-based chemotherapy (HR: 0.88 [95%CI: 0.51-1.49]). Furthermore, the 1-year ATE-risk was increased in univariable analysis in patients with higher cancer stages and higher baseline levels of tumor markers (LDH, AFP, B-HCG), however, these associations did not prevail upon multivariable adjustment for the application of cisplatin-based chemotherapy. Details on risk factor explorations for 1-year ATE risk are provided in **Table 3**.

Discussion

In the present cohort study including 1,277 patients with TGCT, we observed a substantial burden of ATE over a long-term follow-up period. Our estimates indicate that approximately 1 out of 8 patients will suffer an ATE within 25 years after the time of a TGCT diagnosis. Importantly, irrespective of patients´ age, we observed an almost 5-fold increase in subsequent risk of all-cause mortality in those suffering an ATE, indicating the identification of a particularly high-risk sub-population among TGCT survivors.

Importantly, the short- and long-term risk profiles of ATE seem to be influenced by distinct underlying patient-, disease-, and treatment-specific risk factors. Treatment type and intensity

in patients with TGCT largely depend on underlying histology and cancer stage, with cisplatin-based chemotherapy as the mainstay of systemic treatment.[6] Regarding short-term ATE risk, defined within our study as the one-year time frame after TGCT diagnosis, the application of cisplatin-based chemotherapy markedly increased ATE risk. Accordingly, a dose-dependent effect towards ATE risk was observed per increase in applied chemotherapy cycles, suggesting a potential pathophysiological role of cisplatin-induced vascular toxicity during this active treatment timeframe.[14,15] Similarly to our observations, in a population-based cohort study including TGCT patients, an excess in cardiovascular mortality confined to the first year after cancer diagnosis was observed in those treated with cisplatin-based chemotherapy, with no observable differences thereafter.[16] Additionally, in another population-based cohort study, cardiovascular death was increased within the first year after platinum-based chemotherapy, yet no differences were observed thereafter, irrespective of treatment modalities.[17] In another observational study including TGCT patients, the risk of cardiovascular disease was strongly enhanced only within the first year after initiation of cisplatin-based chemotherapy, with a subsequently moderately increased risk after >10 years of follow-up.[5]

Regarding long-term ATE-risk in TGCT survivors, we identified underlying cardiovascular comorbidities and risk factors as important contributors. For example, higher age, diabetes, arterial hypertension, smoking, and an elevated BMI were associated with an increased long-term ATE risk in univariable analysis. In multivariable analyses, we identified higher age, a positive history of smoking, and elevated LDH levels as independent predictors for the development of future ATE. In contrast, other cancer- and treatment-specific factors were not associated with the long-term ATE-risk. Higher age and a positive smoking history represent well-known cardiovascular risk factors,[18] whereas the association of LDH with ATE risk is less clear. Several aspects might explain the potential role of LDH as a predictor of long-term ATE risk in this particular patient cohort. First, elevated LDH represents a biomarker of tumor burden in TGCT,[19] which might thereby reflect a surrogate marker for generally more intense anti-cancer treatment approaches, which might in return affect future ATE risks. Second, a higher tumor burden represented by increased LDH levels might have an intrinsic prothrombotic and proatherogenic effect in patients, adding to the long-term ATE risk.[20,21] Third, higher levels of LDH might represent a surrogate marker for the underlying cardiovascular disease risk, irrespectively of the presence of TGCT, as recently a prognostic role of LDH towards future cardiovascular risk was reported in a general population.[22] Additionally, LDH has previously been reported as an independent predictor of thromboembolic events (comprising both venous

and arterial events) in patients with germ-cell tumors, and for risk of venous thromboembolism in patients with TGCT.[23,24]

Moreover, we were able to efficiently stratify patients according to their future long-term risk of ATE by implementing the identified independent predictors of ATE in a simplified, point-based risk-score, assigning +1 point for patients ≥35 years of age at the time of diagnosis, a positive smoking history, and a baseline LDH of ≥250 IU/L, respectively. According to individual risk-scores, patients were classified in low-, intermediate-, and high-risk subgroups, with corresponding highly divergent 25-year ATE rates of 4%, 11%, and 23%, respectively. Internal validation via bootstrapping revealed a good discriminatory performance ability of the developed score. Upon awaited external validation, this score might provide an easily available tool to identify high-risk subgroups of TGCT survivors that might enable individualized cardiovascular prevention strategies.

Synoptically, the high observed burden of ATE during long-term follow-up in TGCT survivors might be explained by several factors. First, anti-cancer treatments exhibit distinct vascular toxicities and thereby increase ATE rates.[25] Previous studies found an increased risk of cardiovascular diseases in TGCT survivors treated with cisplatin-based chemotherapy compared to those treated with surgery alone.[1] Our findings indicate an increase in ATE risk with cisplatin-based chemotherapy refined to the short-term period rather than a long-term effect. Moreover, radiotherapy has previously been reported as cardiovascular risk factor,[26] whereas in our study the ATE risk was not independently associated with radiation exposure. Cisplatin seems to induce endothelial dysfunction, characterized by increased circulating levels of von Willebrand factor and the release of prothrombotic extracellular vesicles *in vitro*, whereas the exact mechanisms *in vivo* still remain unresolved.[15,27] Further, recently, clonal hematopoiesis of indeterminate potential (CHIP) has been proposed as a potential pathophysiological contributor to cardiovascular diseases, with an increased prevalence of CHIP observed in cancer survivors treated with cytotoxic cancer treatments, including chemo- and radiotherapy used in TGCT patients.[21,28,29]

Second, beyond anti-cancer treatments, lifestyle-specific factors and associated comorbidities might affect the overall cardiovascular disease burden in TGCT survivors. For example, a recent nationwide cohort study found higher rates of adverse health behaviors in TGCT survivors compared to the general population, as represented by higher proportions of smokers and individuals with an elevated BMI.[30] In another study, higher rates of metabolic syndrome were reported in TGCT survivors compared to the general population.[31] Third, metabolic changes due to post-therapeutic hypogonadism in TGCT survivors might lead to the promotion

of pro-atherogenic changes and thereby increased ATE risk.[26] Finally, the cancer itself might exhibit pro-thrombotic and pro-atherogenic effects via shared pathophysiological pathways, such as the induction of systemic inflammation or hypercoagulability,[20,32] contributing to the development of subsequent cardiovascular diseases and ultimately ATE in patients with TGCT. Taken together, our findings support the concept that the long-term risk of ATE in TGCT survivors might in large parts be mediated by underlying patient specific, in part modifiable risk factors, including age and smoking status, whereas the short-term ATE risk seems to be largely affected by the application of cisplatin-based chemotherapy.

Study limitations

Several pertinent limitations to our study need to be acknowledged. First, the retrospective study design might affect the quality of follow-up data. However, due to the standardized follow-up care of patients with regular routine clinical reassessment in the setting of a tertiary academic care center, the severity of our defined endpoints, in combination with data availability of other federal state hospitals, we believe that the completeness and integrity of our collected data, especially regarding outcome variables, is high. Second, in the present study no comparative effect estimates of ATE risk in TGCT patients compared to the general population was feasible due to the lack of an adequate available control group. Therefore, only the absolute burden of ATE in this patient population can be deduced from our findings, whereas the relative risk of ATE in TGCT patients is not provided. However, we believe that the high rate of ATE during our 25-year follow-up period highlights the general risk in this patient population and helps raise awareness. Third, due to the retrospective design of our study, variables included in our risk factor evaluations were limited by data availability. Therefore, novel biomarkers that might aid in stratifying cardiovascular risk profiles in patients, such as growth differentiation factor 15 (GDF-15),[33] genetic alterations including the prevalence of CHIP[28], or epigenomic changes[34] were not evaluated and should be the focus of dedicated studies in the future. Similarly, established cardiovascular risk scores (e.g., SCORE-2, SCORE2-OP) could not be calculated for a sufficient number of patients in our cohort due to the unavailability of individual measurements of systolic blood pressure and levels of cholesterol. Therefore, a systematic comparison of established risk scores in our cohort was not feasible. Fourth, medical comorbidities at study inclusion were defined based on treating physicians and might therefore be subject to limitations based on misdiagnoses. Further, longitudinal changes in terms of various risk factors and health behavior (e.g., smoking cessation, physical exercise, weight loss) were not assessed in the present study and might

further affect future ATE risk. Lastly, decreases in sample sizes over the duration of long-term follow-up led to lower statistical precision for late timepoints in cumulative incidence estimates, which therefore have to be interpreted in the context of their range in confidence intervals.

Conclusion

In conclusion, in our large-scale cohort study including patients with TGCT, we observed a substantial risk of ATE over a 25-year follow-up period, which was independently associated with markedly increased mortality. Cisplatin-based chemotherapy seems to increase short-term ATE risk within one year after cancer diagnosis, whereas baseline patient characteristics, including higher age and smoking seem to increase the long-term ATE risk. A simple point-base risk score efficiently stratifies patients according to their future ATE risk. Future studies should focus on novel biomarkers of cardiovascular risk and longitudinal assessments of ATE risks to improve individualized risk prediction and thereby ultimately enable personalized cardiovascular prevention strategies in TGCT survivors.

Author contributions: FM: Conception and study design, data analysis, drafting of the first manuscript draft. AT: Data collection, interpretation of data, revising of manuscript. MP: Conception and study design, interpretation of data, revising of manuscript. AS, DB, RP, PR, GS, SM, PJ, SA, TB, GH: Interpretation of data, revising of manuscript.

Disclosures: The authors declare no potential conflict of interest.

Funding support: No specific funding was used for the present study.

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Figure legends

Figure 1: Cumulative incidence of ATE after the diagnosis of testicular cancer

Cumulative incidence function and corresponding 95%-confidence interval, accounting for all-cause mortality as competing outcome event. Abbreviations: 95%-CI: 95% confidence interval.

Figure 2: Landmark analysis of overall survival according to occurrence of ATE within 10 years after testicular cancer diagnosis

Abbreviations: ATE: Arterial thromboembolic events

Figure 3: Cumulative incidence of ATE according to a point-based risk stratification score

Patients were assigned +1 point for ≥35 years of age at diagnosis, a positive history of smoking, and baseline LDH values of ≥250 IU/L. Cumulative incidence functions account for all-cause mortality as competing outcome event.

Figure 4: Risk of ATE according to cisplatin-based chemotherapy

Abbreviations: ATE: Arterial thromboembolic events

Tables

Table 1: Baseline characteristics of the study cohort (n=1,277)

Variable	N (% missing)	Median (IQR) / n (%)
Baseline characteristics		
Age (diagnosis)	1,277 (0%)	35 [29-43]
BMI	1,105 (13.5%)	24.9 [22.9-27.5]
BMI (≥25kg/m^2)		543 (49.1%)
BMI (≥30kg/m^2)		142 (12.9%)
Smoker	1,065 (16.6%)	488 (45.8%)
Diabetes mellitus	1,190 (6.8%)	28 (2.4%)
Hypertension	340 (73.4%)	95 (27.9%)
Cancer specifics		
Tumor stage	1,277 (0%)	
- CS I		928 (72.7%)
- CS ≥II		349 (27.3%)
Histology	1,267 (0.7%)	
- Seminoma		714 (56.4%)
- Non-seminoma		553 (43.7%)
Cancer therapy		
Cisplatin-based chemotherapy	1,277 (0%)	557 (43.6%)
- Cisplatin cycles	557	3 [2-4]
- Cisplatin adjuvant		288
- Cisplatin curative		346
Radiotherapy	1,277 (0%)	118 (9.3%)
Biomarkers		
LDH	1,087 (14.9%)	167 [148-198]
- LDH ≥250 IU/L		129 (11.9%)
B-HCG	1,095 (14.3%)	1.2 [1.2-1.2]
- B-HCG ≥5 IU/L		147 (13.5%)
AFP	1,108 (13.2%)	3.1 [2.2-5.4]
- AFP (≥20 ng/mL)		122 (11.0%)

Table Legend: Abbreviations: AFP: alpha-fetoprotein; β-HCG: β human chorionic gonadotropin; BMI: body mass index; CS: clinical stage; IQR: interquartile range; LDH: lactate dehydrogenase.

Table 2: Exploration of risk factors for ATE-risk

Variable	HR for ATE (95%CI)
Baseline characteristics and comorbidities	
Age (per 10 years increase)	**2.17 (1.74-2.71), p<0.001**
Age (≥35 years)	**3.60 (2.01-6.44), p<0.001**
Diabetes mellitus	**10.33 (4.35-24.52), p<0.001**
Smoker	**1.98 (1.09-3.61), p=0.025**
BMI (per unit increase)	1.04 (0.97-1.12), p=0.218
BMI (≥25kg/m^2)	1.05 (0.59-1.88), p=0.861
BMI (≥30kg/m^2)	**2.26 (1.09-4.70), p=0.029**
Cancer specifics	
Stage (CS ≥II vs. CS I)	1.31 (0.78-2.22), p=0.310
Histology (seminoma vs non-seminoma)	1.53 (0.91-2.58), p=0.108
Treatment	
Cisplatin-based chemotherapy	1.15 (0.70-1.99), p=0.591
Cisplatin per cycle increase	1.08 (0.93-1.24), p=0.306
Cisplatin ≥3 cycles vs 0-2 cycles	1.30 (0.77-2.18), p=0.333
Cisplatin (time-dependent)	1.19 (0.67-2.11), p=0.535
Radiotherapy	**2.04 (1.13-3.71), p=0.018**
Biomarkers	
LDH (per double increase)	**1.54 (1.09-2.17), p=0.015**
LDH (≥250 IU/L)	**2.97 (1.46-6.05), p=0.003**
B-HCG (per double increase)	1.02 (0.92-1.14), p=0.707
B-HCG (≥5 IU/L)	1.08 (0.45-2.58), p=0.865
AFP (per double increase)	1.03 (0.90-1.18), p=0.642
AFP (≥20 ng/mL)	0.88 (0.34-2.24), p=0.784

Table Legend: Abbreviations: AFP: alpha-fetoprotein; β-HCG: ATE: arterial thromboembolic events; β human chorionic gonadotropin; BMI: body mass index; CI: confidence interval; CS: clinical stage; HR: hazard ratio; LDH: lactate dehydrogenase.

Table 3: Exploration of risk factors for 1-year risk of ATE

Variable	HR for ATE (95%CI)	Adjusted HR (95%CI)*
Baseline characteristics and comorbidities		
Age (per 10 years increase)	1.00 (0.50-2.01), p=0.998	/
Age (≥35 years)	0.68 (0.15-3.06), p=0.620	/
Diabetes mellitus	n.e. (all events in non-diabetes group)	/
Smoker	3.59 (0.37-34.45), p=0.267	/
BMI (per unit increase)	0.97 (0.93-1.02), p=0.272	/
BMI (≥25kg/m^2)	0.70 (0.12-4.17), p=0.692	/
BMI (≥30kg/m^2)	1.74 (0.20-15.63), p=0.612	/
Cancer specifics		
Stage (CS ≥II vs. CS I)	**6.72 (1.30-34.63), p=0.023**	1.96 (0.38-10.08), p=0.423
Histology (seminoma vs non-seminoma)	0.31 (0.06-1.59), p=0.159	/
Treatment		
Cisplatin-based chemotherapy	**n.e. (all events in cisplatin group)**	/
Cisplatin per cycle increase	**1.38 (1.10-1.74), p=0.006**	/
Cisplatin ≥3 cycles vs 0-2 cycles	3.32 (0.74-14.82), p=0.116	/
Radiotherapy	n.e. (all events in non-radiotherapy group)	/
Biomarkers		
LDH (per double increase)	**1.98 (1.03-3.81), p=0.040**	1.42 (0.67-3.01), p=0.362
LDH (≥250 IU/L)	4.98 (0.83-29.79), p=0.079	1.79 (0.30-10.72), p=0.523
B-HCG (per double increase)	**1.18 (1.01-1.37), p=0.033**	1.09 (0.92-1.29), p=0.315
B-HCG (≥5 IU/L)	**9.72 (1.62-58.15), p=0.013**	3.54 (0.59-21.21), p=0.166
AFP (per double increase)	**1.32 (1.09-1.59), p=0.004**	1.19 (0.97-1.46), p=0.088
AFP (≥20 ng/mL)	5.40 (0.90-32.33), p=0.065	1.91 (0.32-11.49), p=0.475

Table Legend: * Significant Variables from univariable analysis adjusted for cisplatin-based chemotherapy; Abbreviations: AFP: alpha-fetoprotein; β-HCG: ATE: arterial thromboembolic events; β human chorionic gonadotropin; BMI: body mass index; CI: confidence interval; CS: clinical stage; HR: hazard ratio; LDH: lactate dehydrogenase.