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Mortality Risk factors and SOX2 and mTOR expression in Patients with Esophageal Cancer

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ABSTRACT

Causes of mortality in EC patients are not confined to cancer-specific mortality but include various protein expressions of SOX2 and mTOR in Esophageal Cancer patients and their correlation with the clinical stage. Data about the risk factors and involvement of cancer-specific protein are still lacking. This study aimed to define the risk factors and association of SOX2 and mTOR expression in mortality in patients with EC. We conducted a retrospective cohort study to assess the risk factors for cancer-specific mortality and cardiovascular mortality in patients with esophageal cancer (EC). The expression rates of SOX2, as well as MTO, were checked in patients. The multivariate analysis revealed a high-risk EC mortality with age ≥ 65 years, black race, grade, stage, and sequence of treatment; radiation after surgery; radiation before and after surgery; Surgery both before and after radiation. While the cardiovascular mortality increased with age ≥ 65 years, adenocarcinoma type, grade, stage, and sequence of treatment. The expression rates of SOX2, as well as mTOR, were 75.5 percent and 86.8 percent in Esophageal Cancer, while were 10.7 percent and 7.5 percent in osteochondroma, respectively, which was statistically significant ($P<0.05$). Risk factors for cancer-specific mortality and cardiovascular mortality in EC patients include older age at diagnosis, male sex, non-married status, grade III of the tumor, the regional or distant spread of the tumor, no cancer-directed therapy. The expression levels of SOX2, mTOR, and the total survival time were related to the different stages. It shows an upward trend for the expression levels of mTOR and SOX2 in Esophageal Cancer tissues. The expression levels of SOX2 and mTOR are related to the clinical stage, metastasis, and prognosis.

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Introduction

Esophageal cancer (EC) is one of the most common and fatal cancers (1). In 2018, more than 572,000 new cases and 508,000 deaths of EC occurred, representing about 3.2% of the new cancer cases and 5.3% of cancer deaths globally 2. EC is the sixth commonest cause for cancer-specific mortality (2, 3). EC prognosis is related to its stage at diagnosis, but it is usually diagnosed at late stages due to its aggressive nature and the lack of a reliable screening method (4). EC five-year survival rate ranges from 15% to 25% (5), but in the case of early diagnosis, the five-year survival rate exceeds 80% (6).

Histologically, EC is classified into squamous cell carcinoma SCC, adenocarcinoma and other less common subtypes as sarcomas, melanomas, carcinoids, lymphomas, and small cell carcinomas (7). The prevalence of EC and its subtypes vary widely across different geographic regions (8). SCC is the predominant histological type worldwide, especially in China and other Asian countries. However, adenocarcinoma predominates in the USA and western Europe, moving SCC to the second position (2, 8). The relative control of SCC risk factors may be responsible for the predominance of adenocarcinoma in western countries (9). In contrast, the incidence of adenocarcinoma is increasing with the increase of its risk factors in recent decades (10, 11).

Risk factors for SCC include mainly smoking and alcohol consumption with other less evident factors including hot beverages as tea and coffee, genetic factors and low socioeconomic level (12). Men are three times more liable to SCC than women (13). SCC is more frequent than adenocarcinoma in black people and white women, while adenocarcinoma is more frequent in white men (14). Risk factors for adenocarcinoma include gastroesophageal reflux disease GERD, obesity and tobacco smoking (12). In

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cases of GERD, adenocarcinoma usually develops on top of Barrett's esophagus, which is pre-neoplastic columnar metaplasia of the esophageal epithelium that occurs in about 5% to 8% of GERD patients (15, 16). Barrett's esophagus patients develop adenocarcinoma with an annual rate of about 0.5% (17). Adenocarcinoma is eight times more incident in men than in women and five times in whites than in blacks in the USA (12). Now, although there is no specific study on the pathological characteristics of esophageal cancer patients and SOX2 and mTOR, there are reports on the immunolocalization of AQPs in the intact and early degenerative regions of human knee cartilage.

Causes of mortality in EC patients are not confined to cancer-specific mortality but include circulatory, digestive, and respiratory causes (18, 19). Data about the risk factors for cancer-specific and cardiovascular mortality are still lacking. This study aimed to define the risk factors associated with cancer-specific mortality and cardiovascular mortality in patients with EC.

Materials and methods

Study Population

Based on the Surveillance, Epidemiology, and End Results SEER database, we conducted this retrospective cohort study in patients with EC. We extracted the data of all types of EC, including the treatment field from 1975 to 2016, using the SEER*Stat version 8.3.8.

Information Collection

We executed these outcomes from the SEER database; I cancer-specific mortality, II cardiovascular mortality including atherosclerosis, cerebrovascular disorders, aortic aneurysm with dissection, hypertension without heart disease, and the diseases of the heart, the arteries, the capillaries and the arterioles. III survivors. Also, We gathered these variables to estimate the risk factor of mortality; I age at diagnosis, II sex, III race, including white, black and others American Indian / Alaska Native, Asian or Pacific Islander, IV marital status, including single, married and others separated, divorced, widowed, unmarried or domestic partner, V type, including SCC, adenocarcinoma and others uncommon types, VI the primary site, including the upper, middle, lower third of the esophagus, VII grade, including I, II, III and IV, VIII stage, including localized, regional and distant, IX treatment field, including chemotherapy, surgery and radiation, X Sequence, including radiation before surgery, radiation after surgery, radiation both before and after surgery, surgery both before and after radiation and no radiation and/or cancer-directed surgery.

Detection of SOX2 and mTOR expression by qPCR

The SOX2 and mTOR expression were detected by qRT-PCR. Total RNA in the joint fluid was extracted and dissolved in 20 μL DEPC water in view of Trizol reagent operation instructions. It was reversely transcribed using a reverse transcription kit: M-MLV 1 μl, Olig (d T) 1 μl, RNA enzyme inhibitor 0.5 μl, d NTPs 1 μl, RNase free water added to 15 μl. It was cultivated at 38°C for 60 min. We took 1 μl c DNA, 85°C for 5 s; the synthesized c DNA was used as a template for qRT-PCR amplification. PCR reaction system was prepared: 10×PCR buffer 2.5 μl, d NTPs 1 μl, upstream and downstream primers 1 μl each, Taq DNA Polymerase 0.25 μl, dd H_2O added to 25 μl; the conditions were as below: pre-denaturation at 95°C for 15 min, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, a total of 35 cycles, extension at 72°C for 15 min. Three multiple wells were supplied for 3 repeated tests, and SOX2 and mTOR took U6 as the internal reference. Afterward, the amplification and melting curves of Real-Time PCR were confirmed, and the relative amount of the target gene was counted in the light of the result parameters. The relative quantification of the target gene was assessed by $2^{-\triangle Ct}$.

Statistical analysis

All data were statistically analyzed by SPSS 17.0 (Beijing Bizinsight Information Technology Co., Ltd.). The counting data between the two groups were tested by χ^2 test. The measurement data were expressed as (x±s) and compared by independent-samples T-test. The diagnostic value of SOX2 and mTOR for esophageal cancer was evaluated by the ROC curve. The logistic regression model was established with the two as independent variables. The area under the ROC curve of joint detection was fitted by the probability value in the model. $P<0.05$ was

seen as a statistically remarkable difference. We analyzed the variables as univariate and multivariate using proportional hazards regression. The relationships among categorical variables were observed using Pearson's chi-square test. The P-value of less than 0.05 means the analysis showed a significant difference.

Results

Clinicopathologic Characteristics

We listed an overall 29,084 patients with EC in our cohort study, with a mean age of 63.84 years. All general data of patients were compared, and the differences of age, gender, smoking, drinking, medical history, registered permanent residence and educational background between them were not statistically obvious. The total survival patients, EC mortality and cardiovascular mortality were 4015 13.80%, 19770 67.97% and 1367 4.70%, respectively. The chi-square of all variables showed significant difference, $P < 0.001$, except gender, were non-significant, $P = 0.811$. The clinicopathologic features of included patients are shown in Table 1.

Cancer-specific mortality

The HR of EC mortality increased in patients with age ≥ 65 years 1.158, black race 1.191, American Indian and Asian race 1.108, uncommon cancers 1.054, grade II 1.103, grade III 1.314, grade IV 1.314, regional stage 1.185 and distant stage 2.083. Cancer-specific mortality decreased in married patients 0.822 times, adenocarcinoma type 0.881 times, cancer site on the lower third of esophagus 0.917 times, patients underwent chemotherapy 0.678 times, radiation 0.76 times, Surgery 0.512 times, radiation before surgery 0.553 times, radiation after surgery 0.671 times and radiation both before and after surgery 0.542 times (Table 2). The multivariate analysis presented that the EC mortality increased with age ≥ 65 years HR, 1.1; 95% CI, 1.068-1.133, black race HR, 1.094; 95% CI, 1.042-1.148, grade III: HR, 1.253; 95% CI, 1.169-1.343; IV: HR, 1.2; 95% CI, 1.74-1.34, stage regional: HR, 1.467; 95% CI, 1.407-1.529; distant: HR, 1.981; 95% CI, 1.896-2.07 and sequence of treatment radiation before surgery: HR, 1.72; 95% CI, 1.612-1.834; radiation after surgery: HR, 1.368; 95% CI, 1.285-1.457; radiation before and after surgery: HR, 1.763; 95% CI, 1.47-2.114; Surgery both before and after radiation: HR, 2.456; 95% CI, 1.275-4.732. The risk of EC mortality decreased with female HR, 0.923; 95% CI, 0.889-0.959, married states HR, 0.909; 95% CI, 0.872-0.947, adenocarcinoma type HR, 0.912; 95% CI, 0.877-0.949 and treatment field surgery: HR, 0.383; 95% CI, 0.365-0.402; chemotherapy: HR, 0.5; 95% CI, 0.483-0.518; radiation: HR, 0.743; 95% CI, 0.716-0.772 (Table 2).

The risk of mortality caused by cardiovascular diseases increased in patients with age ≥ 65 years, adenocarcinoma type, grade II, III, regional and distant stage, 1.308, 1.123, 1.398, 1.481, 1.366 and 2.415 times, respectively. The cardiovascular mortality reduced in female patients, married states, tumor site on the lower third of esophagus, patients underwent surgery, radiation before surgery, radiation after surgery and radiation both before and after surgery, 0.856, 0.829, 0.739, 0.467, 0.706, 0.696 and 0.522 times, respectively (Table 2). The risk of cardiovascular mortality of EC patients increased with age ≥ 65 years HR, 1.377; 95% CI, 1.219-1.556, adenocarcinoma type HR, 1.154; 95% CI, 1.001-1.331, grade II: HR, 1.247; 95% CI, 1.029-1.512; III: HR, 1.252; 95% CI, 1.029-1.522, stage regional: HR, 1.579; 95% CI, 1.393-1.791; distant: HR, 2.004; 95% CI, 1.687-2.381 and sequence of treatment radiation prior to surgery: HR, 3.14; 95% CI, 2.422-4.07; radiation after surgery: HR, 2.201; 95% CI, 1.687-2.873; radiation before and after surgery: HR, 1.987; 95% CI, 1.026-3.85. The factors associated with low risk of cardiovascular mortality was female patients HR, 0.779; 95% CI, 0.672-0.903, married states HR, 0.779; 95% CI, 0.676-0.945 and treatment with chemotherapy HR, 0.508; 95% CI, 0.436-0.592, radiation HR, 0.532; 95% CI, 0.441-0.641 and surgery HR, 0.208; 95% CI, 0.17-0.255 (Table 2).

Expression of SOX2 and mTOR

The attending physician divided the patients into a good prognosis group (Group A, 40 cases) and a poor prognosis group (Group B, 20 cases) according to the statistics of esophageal Cancer patients after treatment. The SOX2 and mTOR expression levels of group B were higher than those in group B, with statistically significant differences ($P<0.001$). The high expression rates of SOX2 and mTOR protein in esophageal cancer were more in Group B with poor diagnosis and prognosis. Briefly, the high expression

rates of SOX2 and mTOR in esophageal cancer were prominently higher in the control group with χ^2 Value higher than 25 in both cases and p-value lower than 0.001 (Table 3).

Table 3. Comparison of expressions of mTOR and SOX2 in osteosarcoma as well as esophageal Cancer

	SOX2		mTOR	
	High expression	Low expression	High expression	Low expression
Group A (n=40)	7	33	6	34
Group B (n=20)	13	7	14	6
χ^2	25.89		28.143	
P	<0.001		<0.001	

The Relationship between the Expression Level of SOX2 and mTOR with clinicopathological Features in esophageal cancer

The SOX2 and mTOR levels of patients were not related to the age, gender, and location of esophageal Cancer patients, but tied to the disease stage and grade. No diversity in the expression level of SOX2 and mTOR protein in patients with different genders, ages, and histology (Table 4) (P>0.05). The expression rate of mTOR and SOX2 varied depending upon the clinical stage, which was prominently lower in and statistical significance (P< 0.05).

Table 4. Association between the expression level of SOX2 and mTOR to characteristics of various clinical cases

Pathological features	SOX2		χ^2	P	mTOR		χ^2	P
	High expression	Low expression			High expression	Low expression		
Gender								
Male	11	23	3.23	0.137	12	22	2.172	0.759
Female	9	17			8	18		
Age								
>14	12	24	2.958	0.138	13	25	1.322	0.781
≤14	8	16			7	5		
Clinical Stage								
Phase A	15	32	33.581	<0.001	14	31	13.799	<0.001
Phase B	5	8			6	9		
Ability to transfer **w**								
Yes	16	30	18.56	<0.001	15	29	12.162	0.008
No	4	10			5	11		

Diagnostic and predictive value of SOX2 and mTOR in the prognosis of esophageal cancer

Details of univariate analysis are shown in Figures 1 and 2 shows details of multivariate analysis. As to esophageal Cancer patients, the sensitivity, specificity, and AUC of SOX2 and mTOR single diagnosis were 78.75%, 82.14% and 0.863, respectively; the sensitivity, specificity, and AUC values of mTOR single diagnosis were 83.75%, 82.14% and 0.902, respectively. Logistic univariate analysis of risk factors associated with esophageal Cancer metastasis in those patients revealed that there were remarkable differences between patients with poor prognosis and those with good prognosis in terms of disease stage, joint function grade, timely treatment, SOX2 and mTOR (P<0.001). Their disease stage, timely treatment, SOX2 and mTOR were all tied to the prognosis of esophageal cancer and were the risk factors for its prognosis (Figs 1 and 2).

Our analysis included 29,084 EC patients, of whom 19,770 (67.97%) died of cancer-specific causes, and 1367 died of cardiovascular causes. Our results showed that risk factors for cancer-specific mortality and cardiovascular mortality in patients with EC include the age of 65 years or older at diagnosis, advanced grades of the tumor, and regional or distant

spread of the tumor. Protective factors include female sex, married status, chemotherapy, radiotherapy, and surgery. Black race versus white is a risk factor for cancer-specific but not cardiovascular mortality. Adenocarcinoma versus SCC is a risk factor for cardiovascular but protects against cancer-specific mortality. Regarding the sequence of therapy, we found that radiation before surgery, radiation after surgery and radiation before and after surgery have higher risks for cancer-specific mortality and cardiovascular mortality.

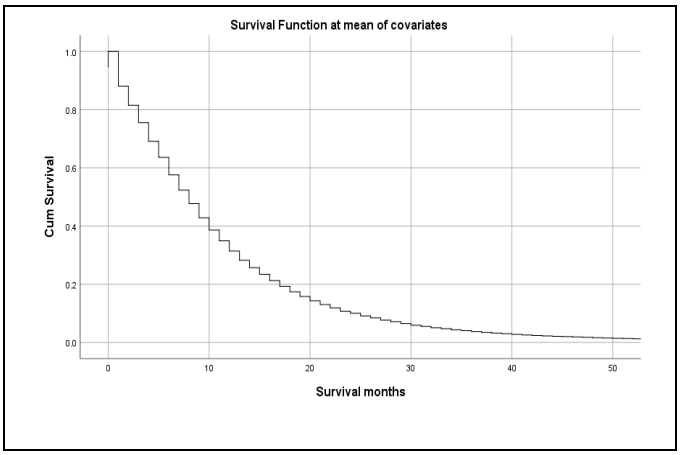

Figure 1. The survival curve for cancer-specific mortality.

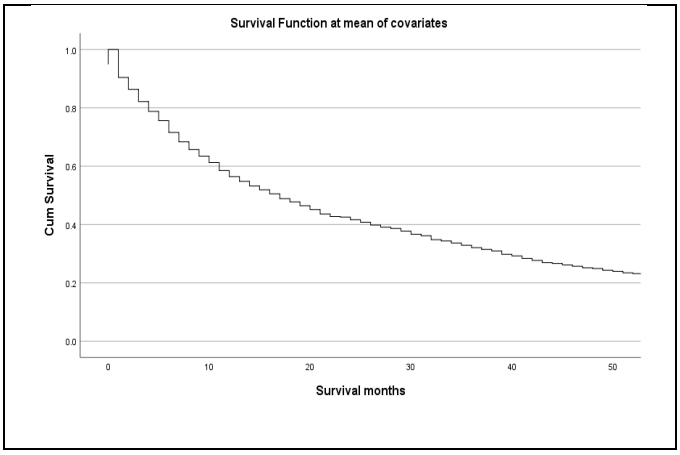

Figure 2. The survival curve for cardiovascular mortality.

Previous studies support the link between advanced age at diagnosis and higher all-cause mortality trends (20, 21) and cardiovascular-specific mortality (22). On the other hand, a recent SEER-based study found that age less than 35 years at diagnosis is associated with higher cardiovascular mortality in cancer patients. However, the study included 28 cancer types, and the results were less representative for EC patients in favor of representing the more common cancer types, especially breast, prostate and bladder cancers that made up most cardiovascular deaths (23). Female sex is associated with lower risks of cancer-specific and cardiovascular mortality in our analysis. Previous studies found that women have more prolonged cancer-specific survival than men (21, 24-26). However, an earlier SEER-based study found no association between sex and cardiovascular mortality. They included the sex variable in the univariate analysis but not included in the multivariate analysis (22).

Regarding cancer differentiation, our results showed that grades III and IV increase the risk of cancer-specific mortality, while grades II and III increase the risk of cardiovascular mortality. Previous studies support the finding that higher grades of the disease are associated with increased mortality risk and lower survival rates (20, 27). However, Rouvelas et al. 2005 revealed that tumor histological variety and histological differentiation are insignificant factors of mortality after surgery (25). This may be because their selected sample was operable and underwent resection surgery, so the prognosis after surgery was less dependent on the histological type and differentiation (25). Earlier studies reported the harmful effect of thorax-directed radiation on the heart (22, 28). They stated that radiotherapy is associated with an increased risk of cardiac toxicity and cardiac death in EC patients (22, 28). In addition, a recent study by Hayashi et al. 2019 stated that these harmful effects are dependent on the level of radiation received by the heart (29). Thus, recent modulations of radiotherapy that adjust the dose and spare the heart may be the cause of our insignificant result (30, 31).

We found no association between tumor site and risk of cancer-specific or cardiovascular mortality. An earlier study found no association between tumor size and mortality in EC patients after surgery (25). On the other hand, the study by Li et al. 2009 on patients with SCC of thoracic esophagus who underwent curative esophagectomy stated that the upper third cancer site is associated with a lower survival rate (27). Other studies found that the lower third cancer site is associated with a higher risk of mortality and cardiovascular mortality (20, 28). This point of disagreement may be related to the surgical procedure

in the study by Li et al. 2009 or more radiation doses directed to the heart in the lower third tumor in the other two studies (20, 27, 28). Amin et al. 2016 found no association between EC-specific mortality with age, race, sex, marital status, size, grade, stage, histological type, and treatment. These insignificant results may be due to their selected sample that included patients with cancer confined to the intraepithelial and mucosal layers only (32). In this regard, further investigation for gene polymorphism (33-37) and a genome-wide association study (38) seem necessary.

We tested SOX2 and mTOR expression differences in esophageal cancer patients and healthy people via qRT-PCR. We found that the SOX2 and mTOR levels in group A were lower than those in group B. AQPs and Toll-like receptors (TLR) affect the biological function of pro-inflammatory cells in related inflammatory diseases, thus affecting the development of arthritis diseases. We believe that SOX2 and mTOR expression with esophageal Cancer is tied to the disease stage and joint function grade of esophageal Cancer. Subsequently, we evaluated SOX2 and mTOR's diagnostic value in esophageal Cancer and its predictive value and found that SOX2 and has good sensitivity and specificity in diagnosing those patients after drawing ROC curves. Logistic univariate analysis and factor analysis revealed that timely treatment, SOX2 and mTOR, were independent risk factors for the prognosis of esophageal cancer patients. However, there has been no previous study on the diagnostic efficacy and predictive value of SOX2 and mTOR expression changes in their joint fluid. SOX2 and mTOR have certain predictive values for the diagnosis and prognosis of esophageal Cancer metastasis patients.

This study relies on high-quality data registered on the SEER database. These data have the advantages of reliable follow-up information and death-related information of EC patients. The results of such an amount of reliable data are more generalizable. In addition, we included many variables that may have affected cancer-specific and cardiovascular mortality. We analyzed data in both univariate and multivariate methods. However, we still have some limitations. Genetic and personal factors that might have affected cancer-specific mortality and cardiovascular mortality are not specified. Besides, different stages of localized malignancy are not distinguished.

Conclusion

Risk factors for cancer-specific mortality in EC patients include older age at diagnosis, male sex, black race, non-married status, grade III or IV of the tumors, the regional or distant spread of the tumor, no cancer-directed therapy. Risk factors for cardiovascular mortality in EC patients include older age at diagnosis, male sex, non-married status, grade II and III of the tumors, Adenocarcinoma subtype, the regional or distant spread of the tumor, no cancer-directed therapy.

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Table 1. Clinicopathologic Features of Esophageal Cancer Patients

Variables	Alive (n = 4015)	Cancer-specific mortality (n = 19770)	Cardiovascular mortality (n = 1367)	Other mortalities (n = 3932)	Total (n = 29084)
Age	61.77 (10.181)	63.93 (10.65)	67.65 (9.612)	64.26 (10.493)	63.84 (10.579)
Sex					
Male	3209 (79.9)	15893 (80.4)	1088 (79.6)	3164 (80.5)	23354 (80.3)
Female	806 (20.1)	3877 (19.6)	279 (20.4)	768 (19.5)	573 (19.7)
Marital status					
Single	584 (14.5)	3067 (15.5)	177 (12.9)	613 (15.6)	4441 (15.3)
Married	2758 (68.7)	11812 (59.7)	818 (59.8)	2406 (61.2)	17794 (61.2)
Other	673 (16.8)	4891 (24.7)	372 (27.2)	913 (23.2)	6849 (23.5)
Race					
White	3538 (88.1)	16354 (82.7)	1141 (83.5)	3322 (84.5)	24355 (83.7)
Black	241 (6)	2336 (11.8)	176 (12.9)	441 (11.2)	3194 (11)
Other	236 (5.9)	1080 (5.5)	50 (3.7)	169 (4.3)	1535 (5.3)
Type					
Squamous cell carcinoma	959 (23.9)	6965 (35.2)	536 (39.2)	1217 (31)	9677 (33.3)
Adenocarcinoma	2770 (69)	11041 (55.8)	743 (54.4)	2338 (59.5)	16892 (58.1)
Other	286 (7.1)	1764 (8.9)	88 (6.4)	377 (9.6)	2515 (8.6)
Site					
Upper third of esophagus	178 (4.4)	1173 (5.9)	76 (5.6)	221 (5.6)	1648 (5.7)
Middle third of esophagus	595 (14.8)	4457 (22.5)	324 (23.7)	768 (19.5)	6144 (21.1)
Lower third of esophagus	3242 (80.7)	14140 (71.5)	967 (70.7)	2943 (74.8)	21292 (73.2)
Grade					
I	446 (11.1)	873 (4.4)	134 (9.8)	293 (7.5)	1746 (6)
II	1911 (47.6)	7448 (37.7)	602 (44)	1600 (40.7)	11561 (39.8)
III	1591 (39.6)	10928 (55.3)	593 (43.4)	1923 (48.9)	15035 (51.7)
IV	67 (1.7)	521 (2.6)	38 (2.8)	116 (3)	742 (2.6)
Stage					
Localized	1659 (41.3)	3480 (17.6)	591 (43.2)	1141 (29)	6871 (23.6)

Regional	1827 (45.5)	7384 (37.3)	533 (39)	1475 (37.5)	11219 (38.6)
Distant	529 (13.2)	8906 (45)	243 (17.8)	1316 (33.5)	10994 (37.8)
Chemotherapy					
No	1352 (33.7)	7379 (37.3)	632 (46.2)	1650 (42)	11013 (37.9)
Yes	2663 (66.3)	12391 (62.7)	735 (53.8)	2282 (58)	18071 (62.1)
Radiation					
No	1576 (39.3)	7916 (40)	588 (43)	1801 (45.8)	11881 (40.9)
Yes	2439 (60.7)	11854 (60)	779 (57)	2131 (54.2)	17203 (59.1)
Surgery					
No	1005 (25)	13110 (66.3)	662 (48.4)	2107 (53.6)	16884 (58.1)
Yes	3010 (75)	6660 (33.7)	705 (51.6)	1825 (46.4)	12200 (41.9)
Sequence					
No radiation and/or cancer-directed surgery	2310 (57.5)	15657 (79.2)	1045 (76.4)	3032 (77.1)	22044 (75.8)
Radiation prior to surgery	1400 (34.9)	2379 (12)	194 (14.2)	573 14.6)	4546 (15.6)
Radiation after surgery	254 (6.3)	1599 (8.1)	118 (8.6)	302 (7.7)	2273 (7.8)
Radiation before and after surgery	42 (1)	126 (0.6)	10 (0.7)	22 (0.6)	200 (0.7)
Surgery both before and after radiation	9 (0.2)	9 (0.1)	0 (0)	3 (0.1)	21 (0.1)

All data are presented as n (%) except age presented as mean (standard deviation).

P-value of all variables were significant (< 0.001) except sex was non-significant (= 0.811).

Table 2. Risk Analysis on the Cancer-specific Mortality and Cardiovascular Mortality

Variables	Univariate analysis		Multivariate analysis			
	Cancer-specific Mortality HR (95% CI)	Cardiovascular Mortality HR (95% CI)	Cancer-specific Mortality HR (95% CI)	Regression Coefficient	Cardiovascular Mortality HR (95% CI)	Regression Coefficient
Age, reference (< 65)						
≥ 65	1.158 (1.126-1.191)[c]	1.308 (1.167-1.467)[c]	1.1 (1.068-1.133)[c]	0.096	1.377 (1.219-1.556)[a]	0.32
Sex, reference (Male)						
Female	1.034 (0.998-1.071)	0.856 (0.75-0.977)[a]	0.923 (0.889-0.959)[c]	-0.08	0.779 (0.672-0.903)[b]	-0.25
Marital status, reference (Single)						
Married	0.822 (0.79-0.856)[c]	0.829 (0.705-0.976)[a]	0.909 (0.872-0.947)[c]	-0.096	0.799 (0.676-0.945)b	-0.224
Other	0.985 (0.942-1.031)	1.022 (0.855-1.223)	0.977 (0.933-1.024)	-0.023	0.944 (0.784-1.137)	-0.058
Race, reference (White)						
Black	1.191 (1.141-1.244)[c]	1.062 (0.906-1.245)	1.094 (1.042-1.148)c	0.09	1.03 (0.863-1.231)	0.03
Other	1.108 (1.042-1.179)[b]	1.141 (0.859-1.514)	1.034 (0.971-1.102)	0.034	1.017 (0.757-1.365)	0.017
Type, reference (Squamous cell carcinoma)						
Adenocarcinoma	0.881 (0.855-0.908)[c]	1.123 (1.004-1.256)[a]	0.912 (0.877-0.949)[c]	-0.092	1.154 (1.001-1.331)[a]	0.143
Other	1.054 (1-1.11)[b]	1.055 (0.842-1.322)	1.018 (0.961-1.078)	0.017	1.061 (0.83-1.357)	0.059
Site, reference (Upper third of esophagus)						
Middle third of esophagus	0.998 (0.936-1.064)	0.792 (0.616-1.018)	1.028 (0.964-1.097)	0.028	0.967 (0.749-1.248)	-0.033
Lower third of esophagus	0.917 (0.864-0.973)[b]	0.739 (0.585-0.935)[a]	1.004 (0.941-1.071)	0.004	0.871 (0.676-1.121)	-0.138
Grade, reference (I)						
II	1.103 (1.028-1.183)[b]	1.398 (1.158-1.687)[c]	1.063 (0.991-1.14)	0.061	1.247 (1.029-1.512)[a]	0.221
III	1.314 (1.227-1.408)[c]	1.481 (1.227-1.787)[c]	1.253 (1.169-1.343)[c]	0.225	1.252 (1.029-1.522)[a]	0.224
IV	1.314 (1.178-1.464)[c]	1.018 (0.709-1.46)	1.2 (1.74-1.34)[b]	0.182	0.868 (0.591-1.276)	-0.141

Stage, reference (Localized)						
Regional	1.185 (1.138-1.234)[c]	1.366 (1.214-1.537)[c]	1.467 (1.407-1.529)[c]	0.383	1.579 (1.393-1.791)[c]	0.457
Distant	2.083 (2-2.168)[c]	2.415 (2.073-2.813)[c]	1.981 (1.896-2.07)[c]	0.684	2.004 (1.687-2.381)[c]	0.695
Chemotherapy, reference (No)						
Yes	0.678 (0.659-0.698)[c]	0.953 (0.857-1.061)	0.5 (0.483-0.518)[c]	-0.693	0.508 (0.436-0.592)[c]	-0.678
Radiation, reference (No)						
Yes	0.76 (0.739-0.782)[c]	0.993 (0.891-1.106)	0.743 (0.716-0.772)[c]	-0.297	0.532 (0.441-0.641)[c]	-0.631
Surgery, reference (No)						
Yes	0.512 (0.496-0.528)[c]	0.467 (0.418-0.521)[c]	0.383 (0.365-0.402)[c]	-0.96	0.208 (0.17-0.255)[c]	-1.569
Sequence, reference (No)						
Radiation prior to surgery	0.553 (0.529-0.577)[c]	0.706 (0.605-0.823)[c]	1.72 (1.612-1.834)[c]	0.542	3.14 (2.422-4.07)[c]	1.144
Radiation after surgery	0.671 (0.637-0.706)[c]	0.696 (0.575-0.842)[c]	1.368 (1.285-1.457)[c]	0.313	2.201 (1.687-2.873)[c]	0.789
Radiation before and after surgery	0.542 (0.455-0.646)[c]	0.522 (0.28-0.975)[a]	1.763 (1.47-2.114)[c]	0.567	1.987 (1.026-3.85)[a]	0.687
Surgery both before and after radiation	0.68 (0.354-1.307)	-	2.456 (1.275-4.732)[b]	0.899	-	-

CI = confidence interval; HR = hazard ratio.

[a]P < 0.05. [b]P < 0.01. [c]P < 0.001