

# Squamous Differentiation in pT1 Low-Grade Bladder Urothelial Carcinoma is Associated with High Risk of Recurrence

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## Research article

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# Abstract

**Background** A large number of studies have confirmed that squamous differentiation is closely related to the prognosis of bladder cancer, and studies also showed that it is an independent predictor for the prognosis of such disease. However, the role of squamous differentiation in pT1 low-grade bladder cancer (LGBC) has not been reported yet. This study mainly intends to explore the clinical significance of squamous differentiation in pT1 LGBC.

**Methods** The clinicopathological data of 582 patients with pT1 LGBC from April 2007 to December 2018 were retrospectively analyzed, including 51 cases pathologically diagnosed as pT1 LGBC with squamous differentiation, and 531 cases as pT1 LGBC without squamous differentiation. The clinicopathological characteristics and prognosis of patients in the two groups were compared.

**Results** 51 cases (8.7%) were pathologically diagnosed as pT1 LGBC with squamous differentiation. As compared with non-squamous differentiation, squamous differentiation was closely related to the age of patients ( $P=0.009$ ) and postoperative recurrence ( $P<0.001$ ). Further studies on the pT1 LGBC recurrence group showed that the patients in the squamous differentiation group were more prone to multiple recurrences ( $P=0.033$ ) and invasive recurrence ( $P<0.001$ ) after surgery. The Kaplan-Meier analysis indicated that pT1 LGBC patients with squamous differentiation had shorter recurrence-free survival (RFS) time. The Cox proportional hazard analysis confirmed that squamous differentiation was an independent predictor of RFS time in patients with pT1 LGBC.

**Conclusions** Squamous differentiation is closely related to postoperative recurrence of pT1 LGBC, especially multiple recurrences and invasive recurrence after surgery. Squamous differentiation is an independent predictor of the RFS time in patients with pT1 LGBC.

## Background

The incidence of bladder tumors ranks first among urinary tract tumors, which seriously threatens the health of people all over the world [1,2]. The majority of bladder tumors are urothelial carcinomas [3], and tissue-type variations with squamous differentiation have been found in about 20%-40% of cases [4-7]. The kind of tissue-type variation is closely related to high invasiveness and easy recurrence of tumor [8]. A large number of studies have clarified that squamous differentiation is an important prognostic factor of urothelial carcinoma [9-13], but there is no relevant research on squamous differentiation in patients with pT1 LGBC. Our study intends to clarify the role of squamous differentiation in pT1 LGBC, and to our knowledge, this is the first study focusing on the clinical significance of squamous differentiation in pT1 LGBC.

## Methods

### Patients and specimens

We retrospectively analyzed the clinical and pathological data of 582 patients pathologically diagnosed as pT1 LGBC in our hospital from April 2007 to December 2018. Patients with the history of urothelial carcinoma or associated urothelial carcinoma were excluded. The patients were divided into two groups based on whether they had squamous differentiation according to the pathological diagnosis. Surgical treatment and intravesical chemotherapy were performed to all patients. The indicators for evaluating the disease progression and prognosis of the patients included age, gender, recurrence, lymphatic vascular invasion and squamous differentiation. Cystoscopy was conducted regularly after the operation. Pathological biopsy was performed if abnormalities were found, and the recurrence was judged based on the results of the biopsy: the urothelial carcinoma in the pathological report of biopsy is defined as recurrence, e.g. the second pathological grade higher than the first pathological grade is defined as invasive recurrence. RFS (recurrence free survival) is defined as the time after patients' operation and before the first recurrence of bladder cancer. The pathological classification is based on the WHO 2004 version of the malignant degree classification system of bladder urothelial carcinoma.

## **Pathology**

All surgical specimens were completely presented to a pathologist, and the pathologist reviewed each pathological section one by one, in order to fully evaluate the clinical stage and pathological grade of tumors. Then two pathologists selected three representative sections of each specimen for independent review, to finally determine the pathological results and squamous differentiation. Routine hematoxylin-eosin (H&E) staining was used to diagnose the presence of lymphatic vascular invasion and squamous differentiation. The presence of intercellular bridges or keratinization was a sign of squamous differentiation.

## **Statistical methods**

The study data was statistically analyzed by R (Version 3.6.3). Chi-square test and T-test were used to analyze the relationship between squamous differentiation and clinicopathological characteristics. The Kaplan-Meier method was used to analyze the overall survival time and the RFS time, and the difference analysis was calculated by log-rank test. The Cox proportional hazard analysis was used for the univariate and multivariate analysis. P-value was bidirectional, and when  $P < 0.05$ , the difference is deemed statistically significant.

# **Results**

## **Clinical characteristics**

Clinical and pathological characteristics were presented in Table 1. There were a total of 582 patients pathologically diagnosed as pT1 LGBC, with the average age of 62.9, including 466 males and 116 females, with the ratio of male to female of 4:1. 51 patients (8.7%) were pathologically diagnosed as pT1 LGBC with squamous differentiation. Squamous differentiation had no significant relationship with the gender ( $P=0.159$ ) or lymphatic vascular invasion ( $P=0.286$ ), but it was correlated with the age ( $P=0.009$ ).

Elderly patients were more prone to squamous differentiation. As compared with patients of the group without squamous differentiation, patients of the group with squamous differentiation were more prone to tumor recurrence after surgery (35% VS 11%,  $P < 0.001$ ).

During the follow-up period, a total of 78 pT1 LGBC patients experienced postoperative recurrence. Detailed clinical and pathological characteristics were shown in Table 2. As compared with the group without squamous differentiation, the group with squamous differentiation was more prone to multiple recurrences (56% VS 28%,  $P = 0.033$ ) and invasive recurrence (44% VS 18%,  $P < 0.001$ ) after surgery.

### **Oncological outcome**

The median follow-up time was 58 months (6-136 months). About 4.8% (28 cases) patients died during the follow-up period, and about 13.4% (78 cases) patients had tumor recurrence after surgery. The five-year overall survival rate of patients with pT1 LGBC was 97.8%, that of the group of tumor patients with squamous differentiation was 92.2%, and that of the group of tumor patients without squamous differentiation was 98.3%. There was no significant difference in the overall survival rate between the two groups ( $P = 0.065$ , Fig. 1). However, as compared with the group without squamous differentiation, the group with squamous differentiation had a shorter RFS time ( $P < 0.001$ , Fig. 2) and a shorter median recurrence time (30 VS 37 months). The univariate COX analysis showed squamous differentiation (HR, 2.834; 95% CI, 1.690-4.754;  $P = 0.001$ , Table 3). Lymphovascular invasion (HR, 1.79; 95% CI, 1.137-2.817;  $P = 0.012$ , Table 3) was an important factor to predict the RFS time of patients with pT1 LGBC. The univariate and multivariate Cox analyses confirmed that squamous differentiation (HR, 2.969; 95% CI, 1.765-4.999;  $P < 0.001$ ; Table 3) was an independent predictor of RFS time, but age and gender were not important factors affecting the RFS time.

### **Pathology**

Histological variations in squamous differentiation were observed by pathologists in all the 51 cases. When an obvious intercellular bridge and/or keratinization were observed in tumor cells, it was considered that the tumor cells had squamous differentiation. Its main histological characteristics are: 1. Clusters or nests of large and middle nuclear infiltrating tumor cells, often with nucleoli; 2. Unclear separation of cytoplasmic amphiphilic or eosinophilic cytoplasmic background. HE stained sections were used to confirm the presence of lymphatic vascular infiltration.

## **Discussion**

Mazzucchelli and Bacchi first reported squamous differentiation as an indicator of the severity and prognosis of urothelial carcinoma [14]. Subsequently, in studies on bladder urothelial carcinoma, squamous differentiation has attracted researchers' more and more attention. Recently, several studies have shown that squamous differentiation is an independent prognostic factor of urothelial carcinoma [15-17], consistent with the results of the study of Li and Yu, who demonstrated by univariate and multivariate analyses that squamous differentiation was an independent prognostic factor of pT1

bladder urothelial carcinoma, and was associated with high risk of recurrence and poor prognosis [10]. However, a large number of literatures have proved that squamous differentiation is only one of the factors affecting the prognosis. For example, Xu et al. analyzed 869 patients with TURBT and found that squamous differentiation was only an independent predictor of postoperative recurrence, which was closely related to tumor recurrence and RFS [17]. Minato et al. retrospectively analyzed the clinical and pathological data of 80 patients who had undergone radical bladder cancer resection, and the results showed that squamous differentiation was an independent predictor of the overall survival and RFS time [18].

There is no relevant research report on the role of squamous differentiation in pT1 LGBC. It was found in our study that elderly patients with bladder cancer ( $P=0.159$ ) were more prone to squamous differentiation. As compared with the group without squamous differentiation, the group of pT1 LGBC patients with squamous differentiation was more prone to tumor recurrence after surgery ( $P<0.001$ ). There was no statistical difference between squamous differentiation and the gender of patients ( $P=0.159$ ) or lymphatic vascular invasion ( $P=0.286$ ). Further studies of the clinical and pathological data of 78 patients with recurrence showed that the group of patients with squamous differentiation was more prone to multiple recurrences (56% VS 28%,  $P=0.033$ ) and invasive recurrence (44% VS 18%,  $P<0.001$ ) after surgery. Therefore, squamous differentiation was closely related to postoperative recurrence of patients with pT1 LGBC, and was an important factor affecting postoperative recurrence of patients.

The study of Li et al. confirmed that squamous differentiation is an important indicator to predict the sensitivity of chemotherapy in the bladder, which is closely related to the insensitivity and poor prognosis of chemotherapy [19]. Lin et al. conducted a meta-analysis of a total of 13,284 patients with LGBC from 19 studies, and the results showed that squamous differentiation is not an independent predictor of the prognosis of bladder cancer, but is associated with poor clinicopathological characteristics, high risk of recurrence and poor prognosis [20]. The results of our study support the above conclusions. The five-year overall survival rates of the groups of pT1 LGBC patients with squamous differentiation and without squamous differentiation were 92.2% and 98.3% respectively. There was no significant difference in the overall survival rate between the two groups ( $P=0.065$ , Fig. 1). However, as compared with the group without squamous differentiation, the group with squamous differentiation had a shorter RFS time ( $P<0.001$ , Fig. 2) and a median recurrence time (30 VS 37 months). The results of the univariate COX analysis showed squamous differentiation (HR, 2.834; 95%CI, 1.690-4.754;  $P=0.001$ ; Table 3) is an important factor affecting the RFS time of patients with pT1 LGBC. The Cox proportional hazard model was used for the multi-factor analysis, further confirming the squamous differentiation (HR, 2.969; 95% CI, 1.765-4.999;  $P<0.001$ ; Table 3) is an independent predictor of RFS time.

Sanderson et al. conducted a follow-up analysis of 1069 bladder cancer patients who had undergone radical bladder cancer resection, and the results showed that the median recurrence time was 39.6 (4.8-112 months) months [21]. Mariappan et al. conducted a 25-year follow-up of 115 patients with bladder cancer, and the results showed that the majority of patients with bladder urothelial carcinoma recurring within 5 years after surgery, and 12% of them had disease progression [22]. Our follow-up results are

consistent with the results of the study of Sanderson and Mariappan. Analysis of the follow-up results of 78 patients showed that 64 (82%) patients with pT1 LGBC recurrence within 5 years after surgery. The squamous differentiation group had a shorter median recurrence time (30 vs. 37 months), but 18% patients with pT1 LGBC still had tumor recurrence after 5 years. This prompts urologists that patients with pT1 LGBC may need a longer period of close follow-up after surgery.

## Conclusions

This is the first study focusing on the clinical significance of squamous differentiation in patients with pT1 LGBC. Squamous differentiation is closely related to postoperative recurrence of pT1 LGBC, especially multiple recurrences and invasive recurrence after surgery. Squamous differentiation is an independent predictor of the RFS time in patients with pT1 LGBC.

## Abbreviations

LGBC: Low-grade bladder cancer

TURBT: Transurethral bladder tumor resection

RFS: Recurrence free survival

SDN: Squamous differentiation-negative

SDP: Squamous differentiation-positive

## Declarations

### Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Tianjin Medical University. All procedures followed were in accordance with the Helsinki Declaration of 1964 and later versions. Each patient was fully informed of the investigational nature of this study and provided their written, informed consent.

### Consent for publication

All authors have reviewed the final version of the manuscript and agree with its content and submission.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no conflict of interest.

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## **Authors' contributions**

WQW, ZQS, GL and YJN designed the study; JWZ, SLX and KRW collected the study material and patients' information; WQW, JWZ, SLX, KRW, ZQS, GL and YJN assembled and analyzed the data; WQW, JWZ, SLX and KRW drafted the manuscript; all authors read and approved the final manuscript.

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## Tables

Table 1 Association of squamous differentiation with clinical and pathological characteristics.

Characteristic	Total	SDN	SDP	p value
Cases, n (%)	582(100)	531(91)	51(9)	
Mean age (range),years	62.9[14-95]	62.5[14-90]	67.3[37-95]	0.009
Gender, n (%)				0.159
Male	466(80)	429(81)	37(73)	
Female	116(20)	102(19)	14(27)	
Male:Female	4:1	4.2:1	2.6:1	
Lymphovascular invasion, n (%)				0.286
NO	407(70)	368(69)	39(76)	
YES	175(30)	163(31)	12(24)	
Intravesical recurrence, n (%)				<0.001
NO	504(87)	471[89]	33[65]	
YES	78(13)	60[11]	18[35]	

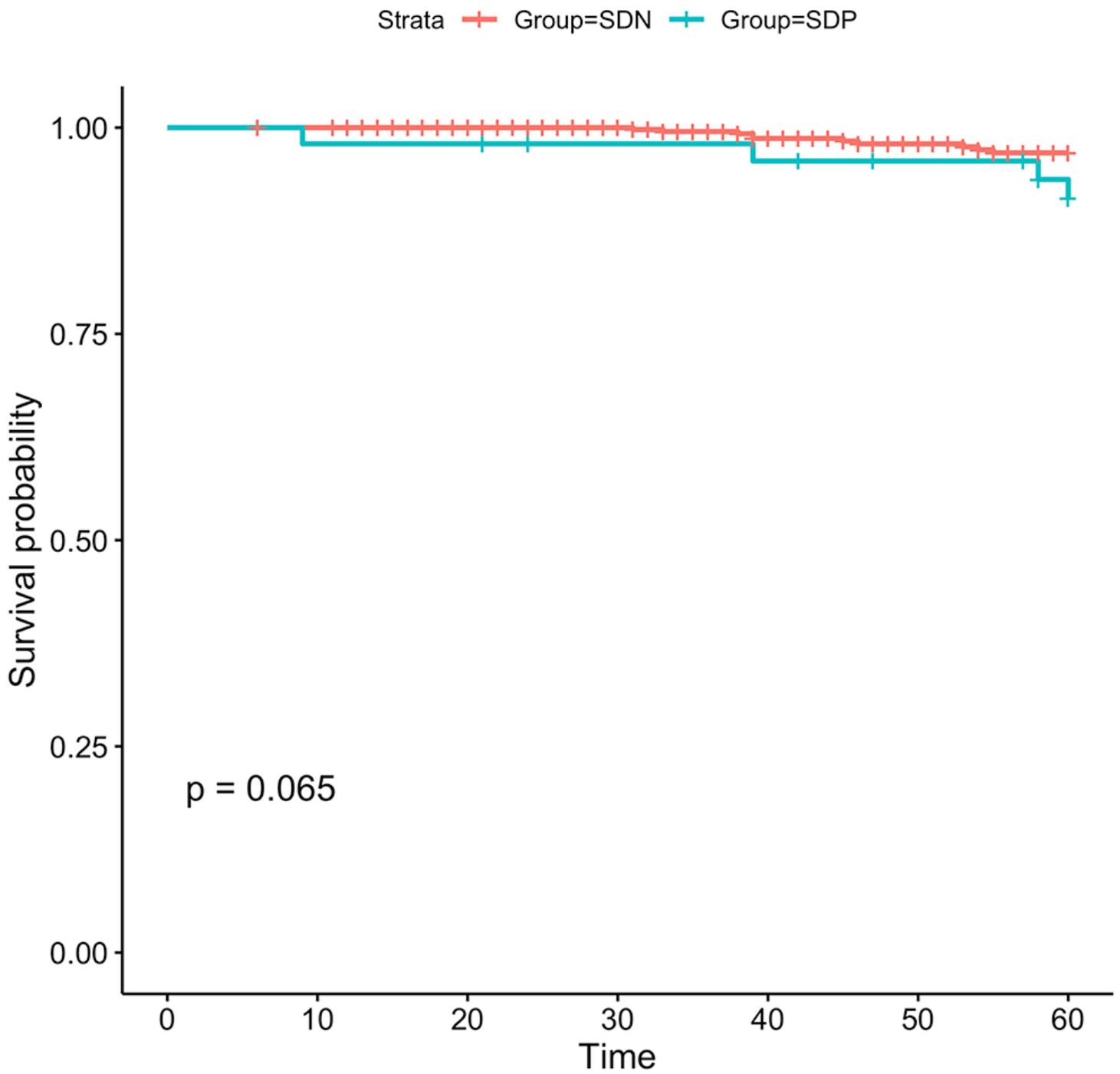
Table 2 Correlation of squamous differentiation with clinical and pathological characteristics in patients experienced postoperative recurrence.

Characteristic	Total	SDN	SDP	p value
Cases, n (%)	78(100)	60(64)	18(23)	
invasive recurrence, n (%)				0.001
Yes	19(24)	11(18)	8(44)	
No	59(76)	49(82)	10(56)	
Number of recurrence, n (%)				0.033
≥2	27(35)	17(28)	10(56)	
=1	51(65)	43(72)	8(44)	

Table 3 Univariate and multivariate Cox regression analyses predicting recurrence-free survival.

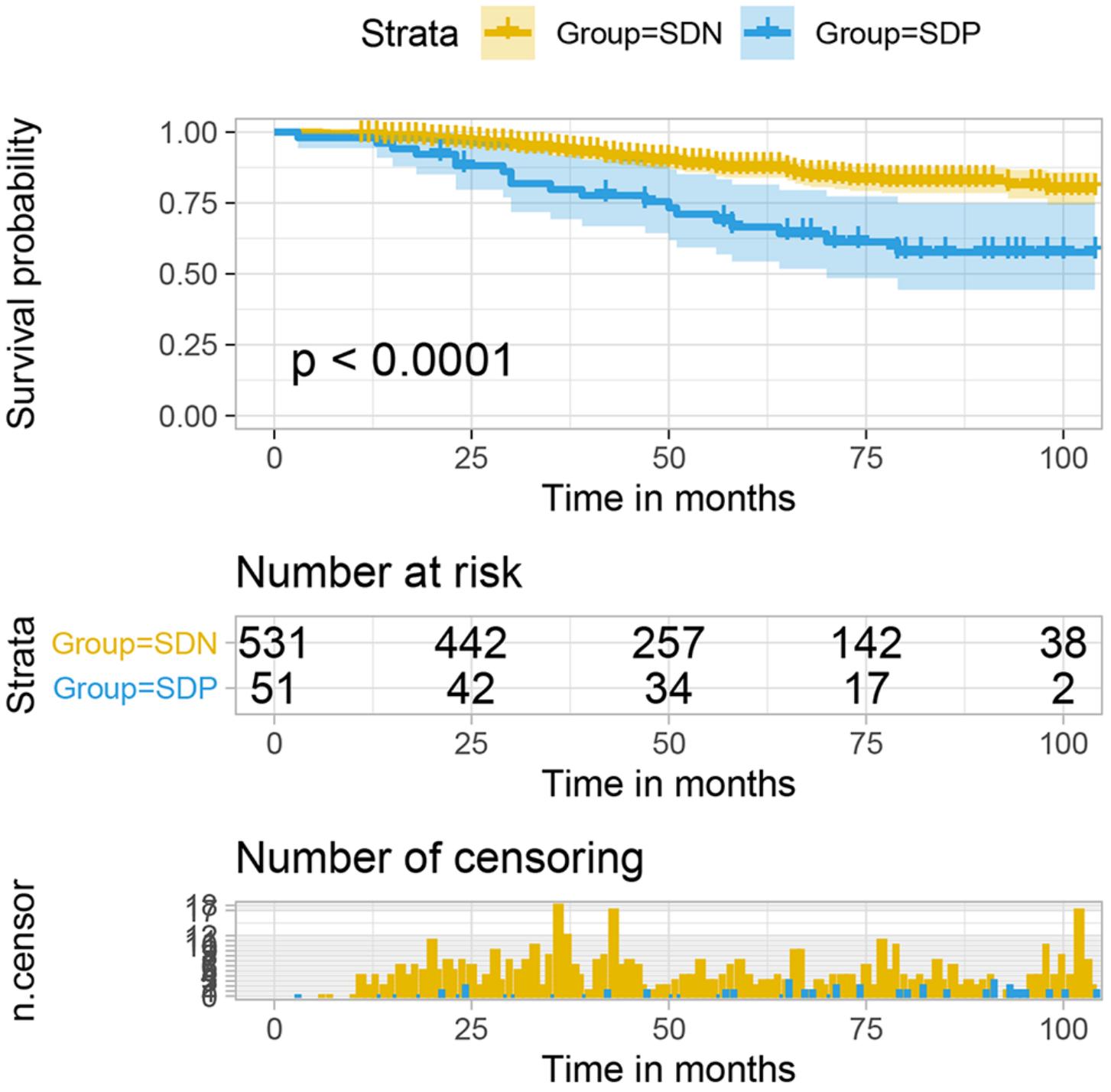
Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age	1.001	0.982-1.019	0.942	0.998	0.981-1.017	0.908
Gender	1.321	0.729-2.395	0.361	1.442	0.793-2.623	0.231
Lymphovascular invasion	1.79	1.137-2.817	0.012	1.836	1.166-2.891	0.009
Squamous differentiation	2.834	1.690-4.754	0.001	2.969	1.765-4.996	<0.001

## Figures



**Figure 1**

Kaplan–Meier curve of the cancer-specific survival rates for the squamous differentiation-negative and squamous differentiation-positive in the subgroup of high-grade ( $p = 0.065$ ). SDN: squamous differentiation-negative; SDP: squamous differentiation-positive.



**Figure 2**

Kaplan–Meier curve of the recurrence-free survival rates for the squamous differentiation-negative and squamous differentiation-positive ( $p < 0.001$ ). SDN: squamous differentiation-negative; SDP: squamous differentiation-positive.