

The association between the histopathological features and microsatellite instability in young patients with urothelial carcinoma of the bladder

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SUMMARY

OBJECTIVE: Bladder cancer under the age of 40 is extremely rare. Bladder cancer development involves complex and multi-stage processes, one of which is the DNA damage repair mechanism. In this retrospective study, we aimed to evaluate the histopathological features of bladder urothelial carcinoma seen in patients under 40 years of age and tumor microsatellite instability status using immunohistochemistry.

METHODS: A total of 50 patients under the age of 40 with urothelial bladder carcinoma from two different centers in the same country were included. Expression of the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2 was analyzed by immunohistochemistry.

RESULTS: Age at the time of diagnosis ranged from 17 to 40 years old. Most tumors were non-invasive papillary urothelial carcinoma. Two cases had nuclear loss of MSH-6 and PMS-2. We observed that tumor grade, tumor stage, presence of tumor differentiation, and infiltrative growth pattern of the tumor have significant impact on prognosis, but microsatellite instability does not have an effective role in bladder carcinogenesis in young patients.

CONCLUSIONS: Our results indicate that the presence of microsatellite instability is not related to the low tumor grade and stage in urothelial neoplasms in young patients, suggesting that urothelial carcinoma of the bladder in young patients may represent a genetically stable form of neoplasia.

KEYWORDS: Urinary bladder neoplasms. Microsatellite instability. Young adult. Carcinoma.

INTRODUCTION

Bladder cancers (BCs) are among the most frequently seen cancers around the world and usually show urothelial carcinoma (UC) morphology¹⁻³. BC is more common in males^{1,4}, and most patients are over 55 years of age. The incidence of bladder cancer under the age of 40 is reported to be 1–2%^{2,5}.

Development of BC involves complex and multistage processes, including oncogenes, tumor suppressor genes, as well as genes involved in recognition and repair of DNA damage⁶⁻¹⁰. Tumor microsatellite instability (MSI) can be defined as the expansion or deletion of one or two repetitive units of

the genomic material and indicates a possible mutation of a mismatch repair gene (MMR)¹¹⁻¹³. DNA damage repair mechanism plays an important role in repairing mutagenic changes in nucleic acids⁶. Normally, the MMR system monitors and corrects base-pair errors within the microsatellite regions^{11,13} as it is one of the main DNA-repair pathways in human cells and maintains genetic stability⁶. The MMR system includes hMLH1, hPMS1, hPMS2, hMSH2, hMLH3, and hMSH6 proteins¹¹. In routine pathology practice, the presence of MMR deficiency, *i.e.*, MSI, is investigated in several cancers. MSI is one of the genetic changes frequently detected in BCs^{3,5}. An increased risk

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of urothelial carcinoma of bladder (UCB) and upper urinary tract has been reported in patients with Lynch syndrome^{8,9}. Moreover, it has been suggested that MSI can be an independent prognostic factor for recurrence risk in UCB¹⁴.

However, to the best of our knowledge, no detailed information is available on MSI status of UCB in young patients. Therefore, in this study, we aimed to evaluate the histopathological features of urothelial carcinoma of bladder (UCB) seen in patients under 40 years of age and tumor MSI status.

METHODS

A total of 50 cases with UCB in patients under 40 years of age diagnosed at two different centers in the same country, between 2000 and 2018, were included. Age, gender, tumor type, grade, stage, presence of necrosis, lymphovascular invasion (LVI), and lymph node metastasis were re-evaluated. Information about the treatment, recurrence and survival status of the cases were retrieved from hospital records.

Expression of the mismatch repair proteins MLH1 (Ventana, M1), MSH2 (Ventana, G219-1129), MSH6 (Ventana, SP93), and PMS2 (Ventana, A16-4) was analyzed immunohistochemically. Immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded tissue microarray (TMA) blocks, using automatic staining system. For the preparation of microarray blocks, hematoxylin-eosin stained slides were reviewed, and tumor areas without necrosis were selected. Two mm core punches from selected areas were used for constructing TMA blocks (Tissue-Tek, Sakura). Normal epithelium was used as positive external control; stromal and inflammatory cells in the tumor sample have been used as positive internal control¹³.

Loss of expression of proteins located in the nuclei of tumor cells was evaluated immunohistochemically. Staining was scored as positive (preserved expression) or negative (loss of expression), according to the percentage of nuclear immune labeling and the staining intensity; cases displaying less than 20% positive tumor cells and/ or very faint staining were classified as negative (MSI phenotype)⁶.

Staining pattern was grouped as follows^{8,9}:

1. Positive: showing nuclear staining in at least some tumor cells;
2. Negative: no nuclear staining with a positive internal control;
3. Cannot be assessed: insufficient technical quality to ensure a definite result despite repeated assays.

Statistical analysis

Statistical analysis was done using the Statistical Package of Social Sciences version 24 (IBM Corp.; Armonk, NY, USA).

P-values less than 0.05 were interpreted as statistically significant. The correlation between overall survival (OS) and disease-free survival (DFS) and age, tumor grade, tumor differentiation, LVI, and necrosis were investigated using Kaplan Meier method and log rank analysis. Multivariate analyses of OS were performed using the Cox regression method.

RESULTS

Patients' ages at the time of diagnosis ranged from 17 to 40 years old, with a median age of 32 years. Only seven cases were of females. Median follow-up time was 48.5 months (min: 4–max: 75, SD: 42.17). Thirty-eight (76%) patients presented with hematuria; six (12%), with dysuria; four (8%), with incidental; and three (6%), with dysuria. Most tumors were non-invasive papillary UC (pTa, n=42, 84%), and the others were pT1 (n=6, 12%), pT2 (n=1, 2%), and pT3 (n=1, 2%).

As to histopathologically, 35 cases (70%) were low grade, 15 cases (30%) were high grade. Most cases (74%) showed papillary growth pattern. Seven cases (14%) had inverted papillary, and four cases (8%) had infiltrative; two cases (4%) had inverted morphology. Three cases showed squamous (two cases) and sarcomatoid (one case) differentiation. Necrosis was observed in three cases. Table 1 demonstrates the histopathological and clinical findings.

All low-grade tumors were non-invasive. Seven of the high-grade tumors were pTa, six were pT1, one was pT2, and one was pT3. There was a statistically significant association between tumor grade and pathologic T stage ($p < 0.001$).

Most low-grade tumors had papillary growth pattern. Infiltrative growth pattern was observed only in high-grade tumors. There was a significant relation between tumor grade and growth pattern ($p < 0.001$).

Necrosis and differentiation were observed only in high-grade tumors. This relation between necrosis and differentiation and tumor grade was statistically significant ($p = 0.023$, $p = 0.023$).

In 48 of 50 patients, no loss of MLH1, MSH2, MSH6, and PMS2 expression was detected (Figure 1). In two cases, high-grade papillary urothelial carcinomas, one non-invasive and the other muscular invasive, had loss of MSH-6 and PMS-2 expression (Figure 2). When the prognoses of these cases were examined, no recurrence was observed in the non-invasive tumor, whereas the tumor showing muscular invasion was observed in the bladder and upper urinary tract 21 months after diagnosis.

One of the patients who died during the follow-up was a 37-year-old male; the other was a 29-year-old female. In both cases, tumor was high-grade, stage was pT1; tumor growth pattern was infiltrative. No tumor necrosis, LVI, and MSI were observed in both cases. One of the cases had tumor differentiation.

When data related to survival were compared, a statistically significant relationship was found between tumor invasion (Figure 3A), pT (Figure 3B), infiltrative growth pattern (Figure 3C), and presence of tumor differentiation (Figure 3D) and overall survival ($p=0.001$, $p=0.002$, $p<0.001$, $p=0.001$). Survival was not associated with tumor accompanying CIS, necrosis, LVI, and MSI ($p=0.762$, $p=0.736$, $p=0.797$, $p=0.762$).

Table 1. Demographic, clinical, surgical, and pathological characteristics of patients.

Median of age (range)	n (%)
	32 (17–40)
Gender	
Female (%)	7 (14)
Male (%)	43 (86)
Symptoms	
Hematuria	38 (76)
Pain	6 (12)
Incidental	4 (8)
Disuria	3 (6)
Pathologic stage	
pTa	42 (84)
pT1	6 (12)
pT2	1 (2)
pT3	1 (2)
Tumor grade	
Low-High	35 (70), 15 (30)
Tumor growth pattern	
Papillary	37 (74)
Papillary + inverted	7 (14)
Inverted	2 (4)
Infiltrative	4 (8)
Tumor differentiation	
Present-absent	3 (6), 47 (94)
Necrosis	
Present-absent	3 (6), 47 (94)
LVI	
Present-absent	2 (4), 48 (96)
Loss of nuclear MLH1 expression	0
Loss of nuclear MSH2 expression	0
Loss of nuclear MSH6 and PMS2 expression	2 (4)

LVI: Lymphovascular invasion

DISCUSSION

Bladder cancer (BC) is the most common cancer seen in the genitourinary tract. Although there are improvements in treatment options, there has been no significant improvement in the survival rates of BCs over the past 30 years¹⁰. Currently, the decision to treat BC is based on the TNM staging system. However, there are significant differences in prognosis of BC patients with the same stage. Today, targeted therapies developed for various tumors and their benefits have directed the researchers to investigate the molecular changes that play a role in the development of BCs.

Microsatellites are repetitive sequences scattered throughout the human genome, consisting of normal nucleotide repeats. Microsatellite regions tend to accumulate mutations because DNA polymerases cannot bind efficiently to these DNA regions during DNA synthesis¹¹. Errors developing in these areas are normally corrected by the mismatch repair (MMR) system^{11,12,15,16}. The MMR system includes hMLH1, hPMS1, hPMS2, hMSH2, hMLH3, and hMSH6 proteins¹¹. MMR deficiency can be detected by IHC, PCR, or new-generation sequencing (NGS)¹⁶. The tumor MSI phenotype determined by PCR is a more specific and “gold standard” method for changes in DNA repair genes, but it has been emphasized that the IHC method can be used as a primary low-cost test^{12,15}. MMR protein analysis by IHC (using four antibodies) and MSI detection by polymerase chain reaction are the first valid screening tests to detect the MMR / MSI phenotype in tumor samples¹³.

Both MSI and low MMR expression have been detected in various cancers, including BC^{7,16}. However, in UCB, very different results have been reported regarding the role of MSI. In the first systematic review, and the study performed by Gonzalez-Zulueta et al.¹⁷, which is one of the largest series published so far, seven different microsatellite genetic changes were investigated in 200 UCB cases. However, data regarding the age of the cases was not available. MSI was detected in six of 200 cases. Since these six cases were pTa or pT1, they claimed that MSI was effective in the early period of bladder tumorigenesis. As a result, the rate of MSI-H detection in UCB cases was reported to be 3%¹⁷.

In their study, Mylona et al.⁷ investigated MSI in the tumor, which included 130 bladder urothelial carcinoma cases, with a median age of 68.9 years. This is the first study to report that hMSH6 protein expression correlates with proliferative and apoptotic indices, hMSH2 and hMSH6 protein expression loss with low-grade noninvasive tumors, and has positive prognostic significance in patients with UC. Catto et al.¹⁸ evaluated the hMLH1 and hMSH2 repair genes in 280 patients with bladder and upper urinary system UC and a mean age of 70 years, and MMR loss was detected by hMLH1 promoter methylation in only one of 117 cases with UCB. They observed that this rate was higher (10%) especially in tumors originating from the upper urinary

system¹⁸. In the study by Vaish et al.¹⁴, involving 44 patients, the mean age of patients was 62 years. The authors concluded that MSI plays an important role in the development and progression of bladder tumors, and the incidence of MSI is higher in patients with high-grade superficial disease. Moreover, they suggested that the high incidence of MSI in superficial tumors that relapse independently of tumor grade may provide an indication for a more radical approach to improving survival¹⁴.

Bladder carcinoma is usually observed at an advanced age and the incidence of BC under the age of 40 has been reported as 1–2 %^{2,5}. Therefore, there are fewer studies evaluating MSI in young cases. In the study by Migaldi et al.², a series of 19 microsatellite loci, previously reported to be associated with BC, were analyzed as for microsatellite changes in a total of 58 superficial (pTa, pT1) UCB subgroups. They reported that microsatellite changes in the form of deletion (LOH) or repetitive sequences are common occurrences in bladder tumorigenesis of young patients, and can be detected at one or more locations in all cancers analyzed. In addition, they reported that microsatellite

changes are more frequently detected in low-grade and low-stage tumors, and suggested MSI as a potential predictor of favorable prognosis². In the study by Wild et al.¹⁹, performed on 14 patients aged 19 or younger with carcinogen-induced urothelial neoplasm (urothelial papilloma (n=1), papillary urothelial neoplasms of low-malignant potential (n=7), pTa low-grade (n=5), pTa high-grade (n=1)), and reported that the UCs are detected rarely in this age group, and they are generally of low grade with a good clinical course. In the same study, they immunohistochemically evaluated the expressions of hMSH2, hMLH1, hMSH6 in the tumor, and did not detect any loss in the expressions of these genes¹⁹. In their study, Giedl et al.⁵ evaluated 204 cases by dividing them into two groups of 17–45 (median age:40) (109 cases) and 50–87 years old (95 cases). Since paraffin-embedded tissue samples were not available in 13 out of 109 cases, immunohistochemical examination could not be performed. MSI-H status was found in one of 109 cases. This case was a 35-year-old patient with T3 BUC. MSI-L was detected in six cases. In two out of 96 cases, loss of MMR protein was detected, as well as

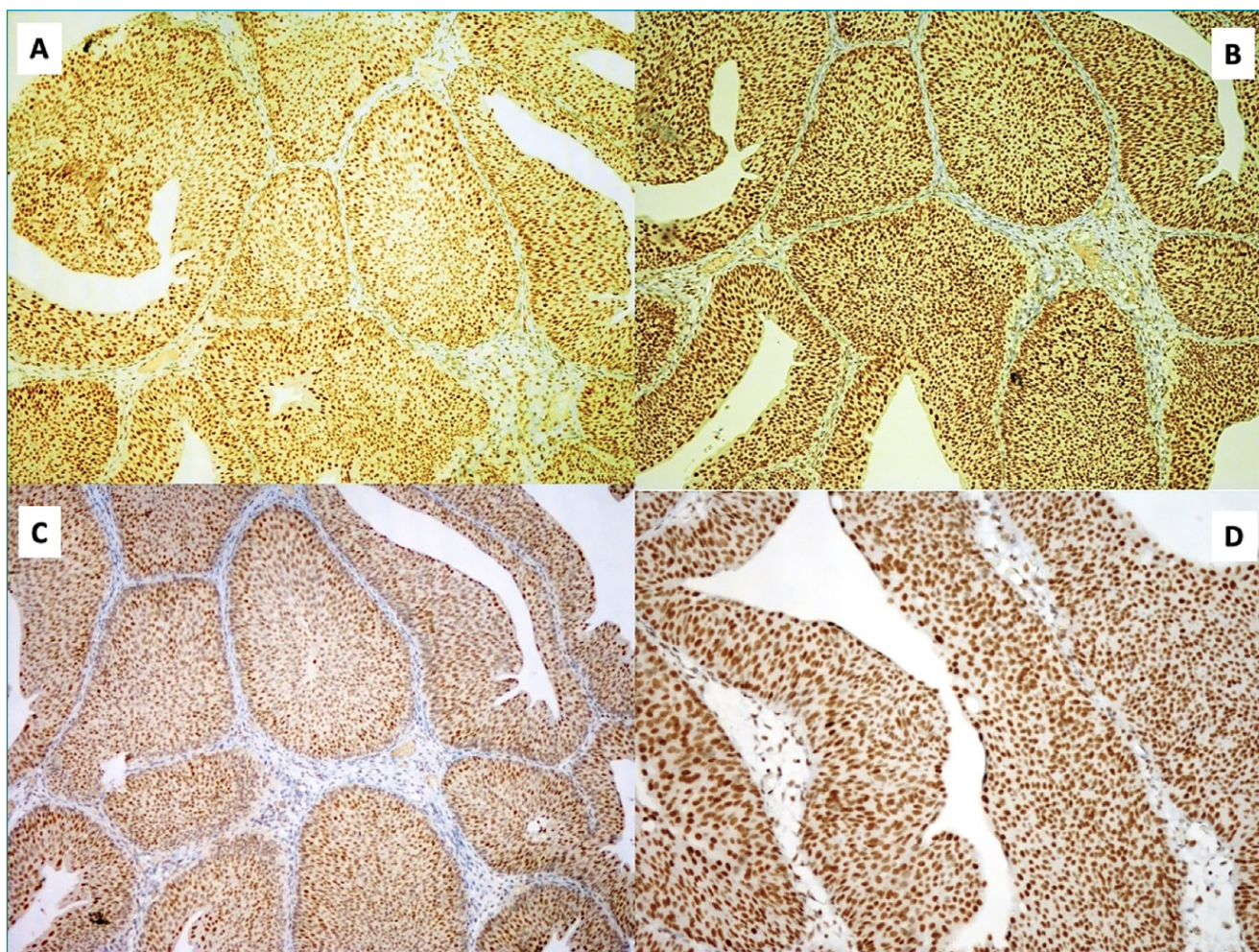


Figure 1. No loss of nuclear expression of MMR proteins (A: MLH-1, B: MSH-2, C: PMS-2, D: MSH-6).

immunohistochemical expression of MSH6. They suggested that the emergence of bladder tumors at a younger age (<45 years of age) is not indicative of MSI⁵. Similar to the results of Giedl et al.⁵, PMS-2 and MSH-6 protein expression loss was observed in only two cases in our study who were both male. Both tumors were high-grade, one was invasive and the other was non-invasive; the tumor showing muscular invasion also had necrosis, squamous, and sarcomatoid differentiation. This supports the fact that the development of BC at a young age cannot be solely explained by the presence of MSI. In addition, in our study, MSI was associated with high-grade tumor, unlike the results reported by Migaldi et al.² and Wild et al.¹⁹, indicating that the presence of MSI was compatible with low-grade and low-stage tumors.

Achievement of different clinical results after treatment in most patients with similar stages and grades, in studies conducted in BC patients with a heterogeneous clinical course, led researchers to investigate new and reliable prognostic factors. Based on various study results, female gender, presence

of tumor differentiation, LNM, necrosis and LVI have been reported as prognostic factors^{4,20-22}. The relation of histopathological features observed in the tumor with overall survival was also investigated. In our study, in addition to tumor grade and stage, tumor differentiation, tumor infiltrative growth pattern and survival in young patients was another relation noted.

The present study has a few limitations. The low number of cases in the study in which the young patient population was included is the most important study limitation. Due to the young age of cases, only two of our cases died with unknown causes, and they were evaluated only in terms of overall survival. Another limitation is that the MSI status in the tumor was only evaluated by immunohistochemistry and could not be correlated with PCR. While designing the study method, evaluating the MSI status with IHC in the first setting, in terms of cost-effectiveness, was planned; and then moving on to the PCR phase with positive cases. However, due to the presence of MSI-H in only two cases in our study, advanced level projecting could not be managed.

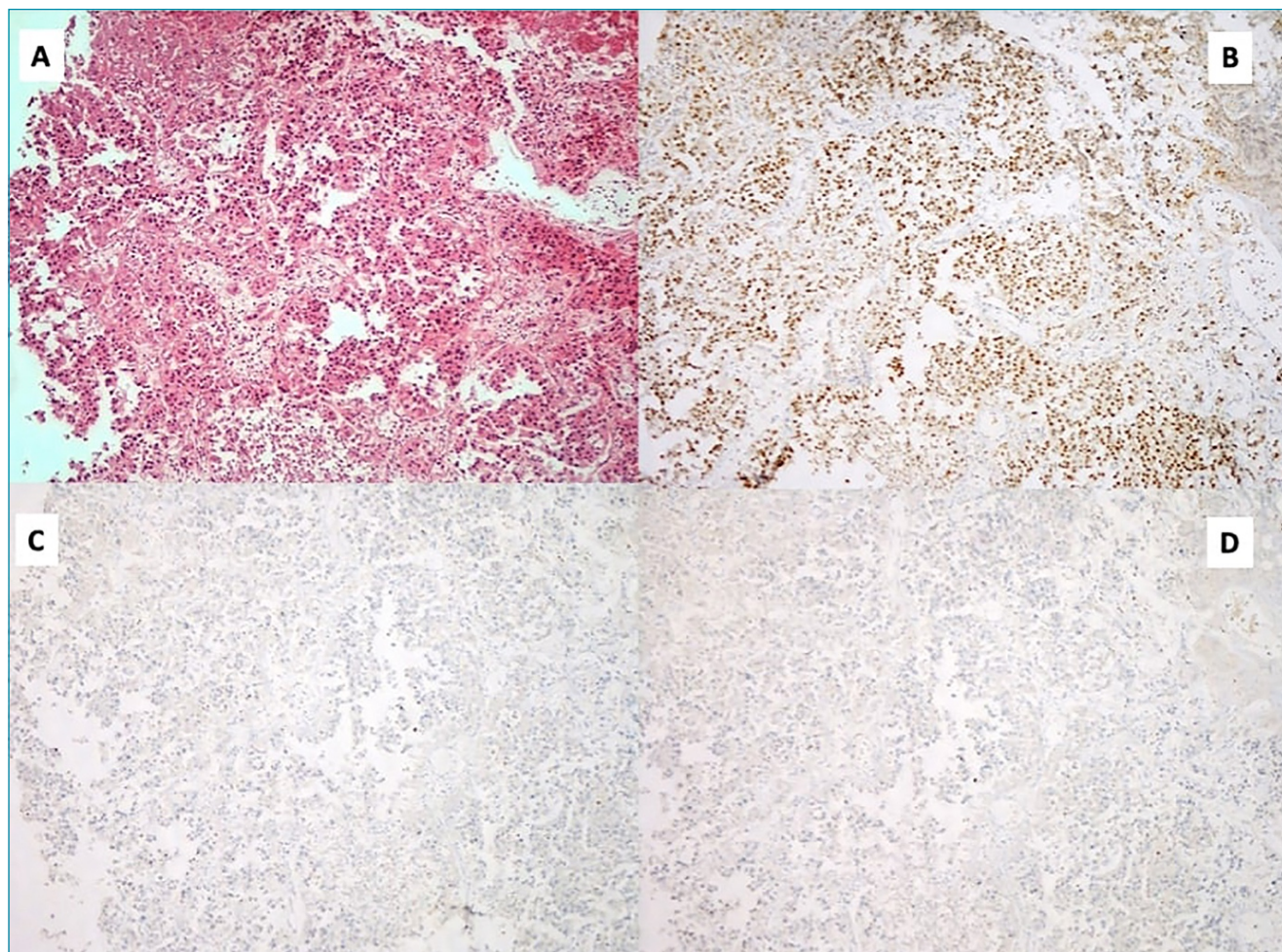


Figure 2. A: Histopathological view of the tumor (H&E, x100), B: No loss of nuclear expression of MSH-2 (DAB, x100), C: Loss of nuclear expression of MSH-6 (DAB, x200), D: Loss of nuclear expression of PMS-2 (DAB, x100).

CONCLUSIONS

Today, targeted therapies applied by determining tumor development pathways are becoming more popular, and they are also being used to make progress in the treatment of BC. The results of the studies on this issue are still controversial. Unlike similar studies, one of the most important features of the present study is that MSI was evaluated with IHC. Another aspect is that the case series is wider than that of other studies evaluating the young age group. When the study results are examined, the most striking point that draws attention is that MSI has no effect on tumor prognosis in young patients. Additionally, unlike the current literature, the presence of MSI was not related to the low tumor grade and stage. In the light of histopathological findings evaluated other than MSI, tumor grade and stage, tumor differentiation and the infiltrative growth pattern of the tumor have prognostic effects in patients with UCB under 40 years of age. Considering the results herein, we suggest that UCB in

young patients should be evaluated as a biologically different and genetically stable group.

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AUTHORS' CONTRIBUTIONS

SE: Conceptualization, Project Administration, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **ÜK:** Conceptualization, Project Administration, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **KY:** Conceptualization, Project Administration, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **ÖK:** . Data Curation, Formal Analysis.

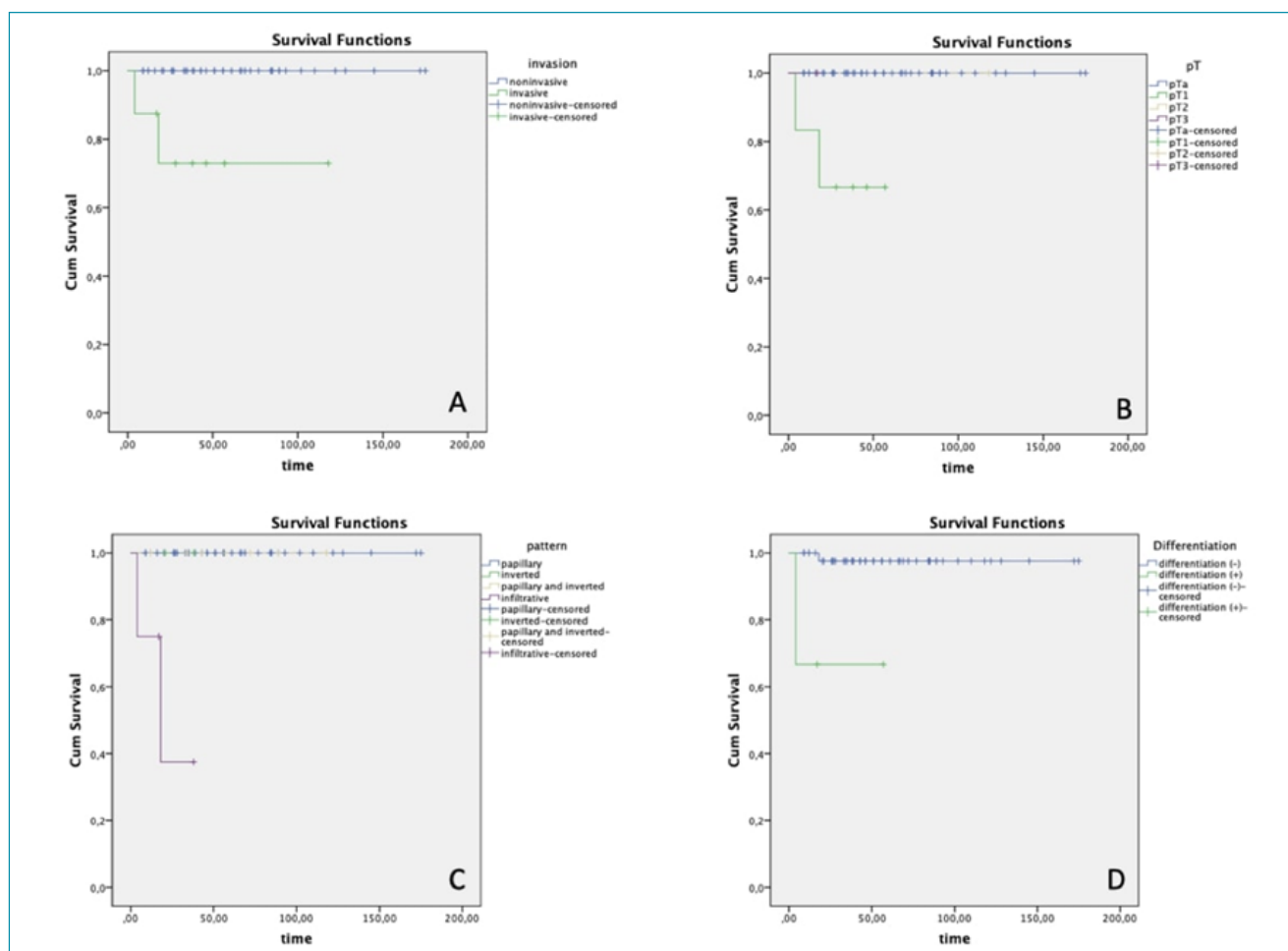


Figure 3. (A) Kaplan-Meier curves of overall survival stratified according to tumor invasion. (B) Kaplan-Meier curves of overall survival stratified according to pathologic T stage. (C) Kaplan-Meier curves of overall survival stratified according to infiltrative growth pattern. (D) Kaplan-Meier curves of overall survival stratified according to the histological differentiation of tumors.

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