Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2020;45(4):1326-1332
DOI: 10.17826/cumj.714836

ARAŞTIRMA / RESEARCH

Importance of cytokeratin-20 expression in papillary urothelial neoplasia

Papiller ürotelyal neoplazilerde sitokeratin-20 ekspresyonunun önemi

Onur Ceylan[1], İbrahim Karabulut[2]

[1] Ataturk University Faculty of Medicine, Department of Pathology, Erzurum, Turkey
[2] SBU Erzurum Regional Training and Research Hospital, Department of Urology, Erzurum, Turkey

Cukurova Medical Journal 2020;45(4):1326-1332

Abstract

Purpose: The aim of this study is to determine the role of Cytokeratin 20 (CK20) expression in the diagnosis of papillary urothelial neoplasms of the bladder, and its relationship with histological grade, recurrence and other prognostic factors.

Materials and Methods: Patients diagnosed with papillary urothelial neoplasm of bladder transurethral resection (TUR) specimens between January 2011 and December 2016 were retrospectively analyzed. Of the 136 selected patients, 32 (24%) were diagnosed with urothelial papilloma, 8 (6%) with papillary urothelial neoplasm of low malignant potential (PUNLMP), 36 (26%) with low grade non-invasive papillary urothelial carcinoma (LGNIPUC), 12 (9%) with high grade non-invasive papillary urothelial carcinoma (HGNIPUC) and 48 (35%) were diagnosed with high grade invasive papillary urothelial carcinoma (HGIPUC). There was no patient diagnosed with LGIPUC. The correlation between CK20 expression and histological grade, tumor recurrence, presence of progression and presence/absence of invasion was investigated.

Results: There was a significant difference between PUNLMP and LGNIPUK in terms of CK20 expression in favor of LGNIPUK. Also there was a significant correlation between histological grade, presence of invasion, recurrence, progression and CK20 expression in patients diagnosed with Papillary Urothelial Neoplasm.

Conclusion: This study supports that CK20 is an important marker in the differentiation between PUNLMP and LGNIPUC, as well as in the determination of histological grade in urothelial carcinomas.

Keywords:. Papillary urothelial neoplasm, CK20 expression, prognostic markers

Öz

Amaç: Bu çalışmanın amacı, mesanenin papiller ürotelyal neoplazilerinde önemli bir immünhistokimyasal belirteç olan Sitokeratin 20 (CK20) ekspresyonunun tanıdaki rolünü, histolojik grade, nüks ve diğer prognostik faktörler ile ilişkisini belirlemektir.

Gereç ve Yöntem: Ocak 2011 ile Aralık 2016 tarihleri arasındaki mesane transüretral rezeksiyon (TUR) spesmenlerine ait papiller ürotelyal neoplazi tanısı almış olgular retrospektif olarak incelendi. Seçilen 136 hastadan 32'si (% 24) ürotelyal papillom, 8'i (%6) malignite potansiyeli belirsiz papiller ürotelyal neoplazi (PUNLMP), 36'sı (%26) low grade non invaziv papiller ürotelyal karsinom (LGNİPÜK), 12'si (%9) high grade non invaziv papiller ürotelyal karsinom (HGNIPÜK) ve 48'i (% 35) high grade invaziv papiller ürotelyal karsinom (HGIPÜK) tanısı almıştı. Low grade invaziv papiller ürotelyal karsinom tanısı alan hasta yoktu. CK20 ekspresyonu ile histolojik grade, tümör nüksü, progresyon varlığı ve invazyon varlığı/yokluğu arasındaki ilişki araştırıldı.

Bulgular: PUNLMP ve LGNİPÜK arasında LGNIPÜK lehine CK20 ekspresyonu açısından önemli fark vardı. Ayrıca Papiller Ürotelyal Neoplazi tanısı alan vakalarda histolojik grade, invazyon varlığı, nüks ve progresyon ile CK20 ekspresyonu arasında anlamlı ilişki bulundu.

Sonuç: Bu çalışma CK20' nin gerek PUNLMP ve LGNİPUK ayrımında gerekse Ürotelyal Karsinomlarda histolojik grade belirlenmesinde önemli bir belirteç olduğunu desteklemektedir.

Anahtar kelimeler: Papiller ürotelyal neoplazm, CK20 ekspresyonu, prognostik belirteçler

Yazışma Adresi/Address for Correspondence: Dr. Onur Ceylan, Ataturk University Faculty of Medicine, Department of Pathology, Erzurum/Turkey E-mail: dr.onurceylan@gmail.com
Geliş tarihi/Received: 10.04.2020 Kabul tarihi/Accepted: 26.08.2020 Çevrimiçi yayın/Published online: 30.12.2020

INTRODUCTION

An average of 260,000 new cases of urinary tract tumors is diagnosed worldwide every year, the majority of which are bladder tumors[1]. Bladder cancer is the fourth most common cancer in men and eighth in women[2-4]. Apart from representing 7% of newly diagnosed cancer cases, its mortality and morbidity are high. Thanks to the advances in the early diagnosis and treatment of bladder cancer, the five-year survival rate has increased to 60-80%[2,3].

According to the classification of the World Health Organization (WHO) and the International Society of Urologic Pathology (ISUP), Non-Invasive Papillary Urothelial Neoplasms are categorized into the subtitles of Urothelial Papilloma, Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP), Low Grade Non-Invasive Papillary Urothelial Carcinoma (LGNIPUC), Low Grade Invasive Papillary Urothelial Carcinoma (LGIPUC), High Grade Non-Invasive Papillary Urothelial Carcinoma (HGNIPUC), and High Grade Invasive Papillary Urothelial Carcinoma (HGIPUC)[1,5,6]. The term of invasion is used when tumor cells cross the basement membrane. The majority of infiltrative (invasive) urothelial carcinomas are high grade. In infiltrative urothelial carcinomas, high- and low-grade tumors are very different in terms of prognosis and therefore, histological grade should be indicated[1]. Low-grade carcinomas have a better prognosis in both invasive and non-invasive tumors compared to high-grade carcinomas[5]. For this reason, it is of prime importance to differentiate these neoplasms well and not to miss a potential malignancy in terms of survival. Sometimes the differentiation of these neoplasms is morphologically challenging, especially in small biopsies including crush artifacts. In such cases, immunohistochemical markers may be helpful in the diagnosis. Especially in recent times, Cytokeratin 20 (CK20) has been investigated in urothelial neoplasms for diagnostic and prognostic purposes.

Cytokeratin is one of the intermediate filament polypeptides found in epithelial cells. CK20, a member of these cytokeratins, is expressed in normal tissues such as intestinal epithelium, urothelium and merkel cells, as well as increasingly expressed in urothelial dysplasias[7-9]. Moreover, in various studies, it has been shown to have an important role in determining the prognosis in urothelial neoplasia[4,7,10,11]. Although the importance of CK20 in determining histological grade has been adequately indicated in the studies in the literature, there is a limited number of studies indicating its prognostic significance. The aim of this study is to investigate prognostic significance of CK20 expression, how the clinician should behave in the presence of CK20 expression, as well as how CK20 immunomarker should be used in cases of diagnostic difficulties.

MATERIALS AND METHODS

In this study, patients diagnosed with Papillary Urothelial Neoplasia of bladder transurethral resection (TUR) specimens which were examined between January 2011 and December 2016 were retrospectively analysed in Medical Pathology Department of Erzurum Regional Training and Research Hospital. The clinicopathological data of the cases were obtained from the automation system of our hospital. In the last 6 years, 199 cases were diagnosed with Papillary Urothelial Neoplasia (Urothelial Papilloma, PUNLMP, LGNIPUC, LGIPUC, HGNIPUC, HGIPUC), but 22 of these paraffin blocks and / or glasses could not be reached. Also only the first biopsies of the repeat biopsies of the same patient were included in the study. There were repeat biopsies in 41 cases and they were not included in the study. Therefore, 136 cases with access to paraffin blocks and glass preparations were included in our study. Sections from the blocks where the tumor was the most intense were investigated by immunohistochemical (IHC) method. Taking 4-micron slices from the blocks where the tumor was present most densely, the tissues placed on charged slides were kept in a drying oven at 70 degrees for 15 minutes and then placed in a Roche Ventana automated immunohistochemistry staining device (Ventana Roche, USA). After the tissues were respectively deparaffinized and dehydrated in the device, they were treated with ULTRA Cell Conditioning Solution, hydrogen peroxidase and CK20 antibodies (Nova Castra, Leica, Newcastle, United Kingdom).

The glass preparations of the patients stained with hematoxylin eosin and CK20 (by immunohistochemical (IHC) method) were reevaluated by us with a light microscope. There was no change in diagnosis in all 136 cases. Thus, the cases were evaluated by two pathologists. The staining characteristic for CK20 was defined as follows: score 1: negative staining or staining of superficial "umbrella" cells only; score 2: focal or

equivocal-patchy or focal thickness staining; score 3: positive - strong and full-thickness staining (Figure 1-3). The relationship between the CK20 expression score and histopathological diagnosis, histological grade, prognostic factors such as recurrence and progression examined. The cases were followed for at least 3 years and recurrent cases were accepted as recurrence and these cases were also analyzed. Cases whose histological grade and / or depth of invasion increased in repeated biopsies were considered as progression.

The study was approved by our local ethics committee (Erzurum Regional Training and Research Hospital 2017 / 37732058-514.10).

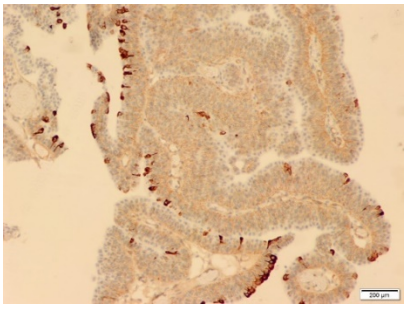

Figure 1. Score 1 Cytokeratin 20 immunostaining

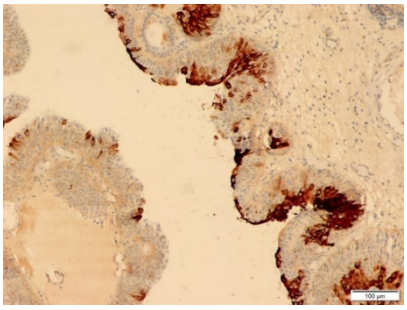

Figure 2. Score 2 Cytokeratin 20 immunostaining

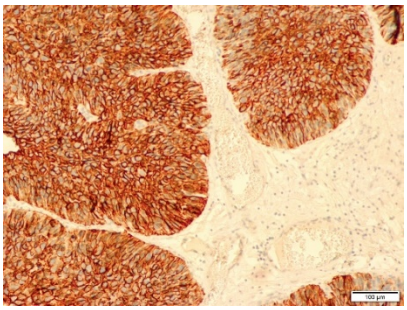

Figure 3. Score 3 Cytokeratin 20 immunostaining.

Statistical analysis

D'Agostino Pearson test was used to determine whether the data fit the normal distribution. Normally distributed binary data groups were compared using independent t test. The Chi square test was used to compare the ordered variables. Pearson correlation was used for correlations between ordered variables. The test was accepted as significant when two-tailed p values were <0.05. Statistical analyses were performed using the Medcalc program (Medcalc ver 16. Ostend, Belgium).

RESULTS

The ages of the patients ranged from 36 to 89 years (mean 73 years) and of the patients, 35 were female and 101 were male. None of the patients had received neoadjuvant therapy. Of the 136 selected patients, 32 (24%) were diagnosed with Urothelial Papilloma, 8 (6%) with PUNLMP, 36 (26%) with LGNIPUC, 12 (9%) with HGNIPUC and 48 (35%) were diagnosed with HGIPUC. There was no patient diagnosed with LGIPUC. Some of the patients diagnosed with papilloma and PUNLMP had completely negative staining for CK20, while the others had positive staining for CK20 only in umbrella cells (Score 1). Of the 36 patients diagnosed with LGNIPUC, 6 (17%) had score 3, 10 (28%) had score 2, and 20 (46%) had score 1 staining with CK20. There was a significant difference in CK20 expression between PUNLMP and LGNIPUC (p: 0.006). Of the 12 patients diagnosed with HGNIPUC, 6 (50%) had score 3 with CK20, 4 (33%) had score 2, and 2 (17%) had score 1 staining with CK20. Of the 48 patients diagnosed with HGIPUC, 42 (88%) had score 3, 2 (4%) had score 2 and 4 (8%) had score 1 staining with CK20. There was a significant correlation between histological grade and CK20 expression in Papillary Urothelial Carcinomas (p:<0.001) (Table-1). Moreover, there was a significant correlation between invasion and CK20 expression when the CK20 expressions of the patients diagnosed with HGNIPUC and HGIPUC were compared (p:0.008).

No recurrence was observed in any of the 8 patients diagnosed with PUNLMP and Papilloma. Recurrence was observed in 16 (44%) of 36 cases diagnosed with LGNIPUC, 5 (41%) of 12 cases diagnosed with HGNIPUC, and 20 (42%) of 48 cases diagnosed with HGIPUC When urothelial carcinomas were compared, no significant correlation was found between histologic grade and recurrence (p:0.397)

(Table-1). Three of 16 patients, diagnosed with LGNIPUC who had recurrence, showed progression. Two of these had progressed to HGIPUC and the other to HGNIPUC. Of the 3 patients with progression, all had Score 3 staining with CK20, and of the other 13 patients with no progression, 2 had Score 3 staining with CK20, 6 had Score 2 staining, and 5 had Score 2 staining. Only one of the 20 patients without recurrence had Score 3 staining with CK20. The other 4 patients had score 2, and 15 patients had score 1 staining. In other words, 5 (31%) of the 16 patients with recurrence had Score 3 staining with CK20, while only 1 (5%) of 20 patients without recurrence had Score 3 staining with CK20.

There was a significant correlation between recurrence, progression and CK20 expression in patients diagnosed with LGNIPUC (p:0.014) (Table-2). Of the 5 patients who were diagnosed with HGNIPUC and had recurrence, 4 had Score 3 staining and one had Score 1 staining with CK20. Progression to HGIPUC was seen in all of the recurrent cases. Of the 7 patients without recurrence, 2 had Score 3, 4 had Score 2, and 1 had Score 1 staining. In other words, of the 5 patients who were diagnosed with HGNIPUC and had recurrence, 4 (80%) had Score 3 staining with CK20, while of the 7 patients without recurrence, 2 (28%) had Score 3 staining. There was no significant correlation between recurrence, progression and CK20 expression in the patients diagnosed with HGNIPUC (p:0.234) (Table-2).

Of the 20 patients who were diagnosed with HGIPUC and had recurrence, 18 had Score 3 staining, the other 2 had Score 2 staining with CK20. None of the patients with recurrence had Score 1 staining. Of the 28 patients without recurrence, 24 had Score 3 and the other 4 had Score 1 staining. In other words, 18 (90%) had of the 20 patients who were diagnosed with HGIPUC and had recurrence had score 3 staining, while 24 (86%) of the 28 patients without recurrence had Score 3 staining with CK20. There was no significant correlation between recurrence and CK20 expression in the patients diagnosed with HGIPUC (p: 0.585) (Table-2).

Table-1. Relationship between histological grade, CK-20 score and relapse rate

	High-Grade PUC (N=60)	Low Grade PUC (N=36)	p value
Median CK20 score	3	1	**<0.001**
Rate of relapse (%)	25 (60)	19 (52.7)	0.397

PUC: Papillary Urothelial Carcinoma, CK20: Cytokeratin 20

Table-2. Relapse status of low- and high-grade tumors and association with CK20 scores

	Relapsed	Non-relapsed	p value
Mean CK20 score for LGNIPUC	2	1	**0.014**
Mean CK20 score for HGNIPUC	3	2	0.234
Mean CK20 score for HGIPUC	3	3	0.585

CK20: Cytokeratin 20, LGNIPUC: Low Grade Non-Invasive Papillary Urothelial Carcinoma, HGNIPUC: High Grade Non-Invasive Papillary Urothelial Carcinoma, HGIPUC: High Grade Invasive Papillary Urothelial Carcinoma

DISCUSSION

Recurrence and progression is not a common finding in Papilloma and PUNLMP, while patients diagnosed with LGNIPUC / HGNIPUC and LGIPUC / HGIPUC that limited to lamina propria have a high recurrence rate and require long-term close clinical follow up and are usually treated conservatively. According to the WHO/ISUP classification system, PUNLMP is classified as neither benign nor malignant neoplasm[1,12,13]. In some studies in the literature, it has been shown that PUNLMP poses a low risk of recurrence and progression and never results in cancer-related death[12,14]. Therefore, accurate diagnosis is of great importance for these patients. Especially, histological differentiation between Papilloma, PUNLMP and LGNIPUC, as well as Low-Grade PUC and High-Grade PUC may sometimes be very challenging and additional immunohistochemical studies may be needed to differentiate them.

In urothelium, CK 20 is normally expressed only in superficial cells, whereas abnormal expression is seen in urothelial dysplasia. CK20 staining is a useful marker to differentiate dysplasia from non-neoplastic hyperplasia and reactive urothelial type. Because of

this feature, CK20 is a biomarker used for differentiation between Urothelial Papilloma, PUNLMP and Urothelial Carcinoma[7,9].

Alrashidy et al. indicated in their study that CK20 immunomarker can be used to demonstrate the change of urothelial cells in the direction of malignancy. They emphasized that this is particularly useful in differentiating between Papilloma and PUNLMP[15]. Van Oers et al. reported that CK20 can only be significant in differentiating between High Grade PUC and Low Grade PUC[16]. None of the cases with the diagnoses of papilloma and PUNLMP included in our study showed abnormal CK20 expression. Abnormal CK20 expression was observed in 45% (Score 2: 10, Score 3: 6) of patients diagnosed with LGNIPUC. In our study, increased CK20 expression (Score 2 or Score 3 staining) was considered significant in the differentiation between LGNIPUC and PUNLMP and papilloma, while normal CK20 expression or negativity (Score 1 staining) was not considered significant in the differential diagnosis. Moreover, unlike the studies in the literature, no difference was found between Papilloma and PUNLMP in terms of CK20 expression, and it was not significant in the differentiation of these two.

A significant correlation between CK20 expression and histological grade, presence of invasion, which are important prognostic factors, has been shown in some studies in the literature. In a study conducted by Mumtaz et al., while abnormal CK20 expression increased up to 68.8% in High Grade PUC patients, this rate was found to be 40.4% in Low Grade PUC patients[17]. Bertz et al. also emphasized that CK20 is an important factor in the determination of biological aggressiveness of Urothelial Carcinomas[18]. Ogata et al. indicated that CK20 is an important predictive factor in determining histological grade[4]. Abdul-Maksoud et al. reported that CK20 is an important prognostic marker in histologic grade and stage[19]. Sikic et al. indicated that CK20 is a biomarker associated with poor prognosis in Urothelial Carcinomas[20].

In our study, increased expression of CK20 (especially Score 3 staining) showed a statistically significant correlation with the presence of invasion and histological grade, similar to the studies in the literature. Therefore, we are of the opinion that if differential diagnosis is torn between Low Grade PUC and High Grade PUC and histopathological views cannot lead us to the definitive diagnosis, especially in cases of diffuse CK20 expression (Score 3), we should first think in favor of High Grade PUC. We think that more serial slices should be taken from TUR materials and examined in more detail because of the risk of invasion in patients with the diagnosis of HGNIPUC and Score 3 staining with CK20. We believe that if we cannot be exactly sure of invasion and we observe score 3 CK20 staining, especially in cases of crush artifacts, it would be appropriate to recommend a rebiopsy as soon as possible in order to determine the invasion status.

Papillary Urothelial Carcinomas have a high rate of recurrence and progression and these are important factors affecting mortality and morbidity. Therefore, the determination of important prognostic factors in recurrence and progression is of great importance. In a study by Harnden et al., it was reported that abnormal CK20 expression was a significant predictive factor for recurrence rate and progression in PUNLMP and Low Grade PUC. Ramos et al. indicated that abnormal CK20 expression was significantly higher in patients with recurrence[11]. Alsheikh et al. found in their study that recurrence rate was higher in patients with abnormal CK20 staining compared to those with normal CK20 staining[7]. Ogata et al. reported that CK20 is an important factor in determining tumor progression and recurrence in addition to histological grade[4]. As same as these studies, there was a strong correlation between recurrence and progression and CK20 overexpression in the patients diagnosed with LGNIPUC in our study. The point to be emphasized is that all patients who were diagnosed with LGNIPUC and showed progression had Score 3 staining with CK20. Although the correlation between CK20 expression could not be investigated in our study due to the absence of recurrence in the patients diagnosed with PUNLMP, the tendency towards malignant transformation in patients with PUNLMP has been found to be higher in those with abnormal CK20 expression studies in the literature [15]. For this reason, it is important to study CK20 and closely follow up patients with overexpression in both PUNLMP and Urothelial Carcinoma cases. We are of the opinion that follow-ups, and if necessary, rebiopsies should be performed at shorter intervals due to the risk of progression, especially in patients with the diagnosis of LGNIPUC and Score 3 staining with CK20. For this, we think that it is not enough to report as only "increased CK20 expression" while indicating CK20 expression in pathology reports and it should be standardized. In our study, there was no

significant link between CK20 overexpression and recurrence and progression in patients diagnosed with HGNIPUC and HGIPUC. This is attributed to the observation of Score 3 CK20 staining in the majority of patients diagnosed with High Grade PUC. CK20 is an important biomarker in predicting recurrence and progression, especially in LGNIPUC. Especially in cases with Score 3 staining with CK20, a significant degree of recurrence and progression is seen compared to the others. Therefore, we recommend that the CK20 staining score be stated in the pathology reports and a scoring system should be used if necessary.

In conclusion, this study supports that CK20 is an important marker in the differentiation between PUNLMP and LGNIPUC, as well as in the determination of histological grade in Urothelial Carcinomas. Moreover, it shows the importance of following up the patients with CK20 overexpression at shorter intervals since a significant correlation was found between CK20 expression and recurrence and progression.

Yazar Katkıları: Çalışma konsepti/Tasarımı: OC, İK; Veri toplama: OC; Veri analizi ve yorumlama: OC; Yazı taslağı: OC, İK; İçeriğin eleştirel incelenmesi: OC, İK; Son onay ve sorumluluk: OC, İK; Teknik ve malzeme desteği: İK; Süpervizyon: OC; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için yerel etik kurulumuz tarafından onaylanmıştır (Erzurum Bölge Eğitim ve Araştırma Hastanesi 2017/37732058-514.10).
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.
Yazarın Notu: XVII. Çukurova Pediatri Günleri, Çukurova Pediatri Hemşireliği ve Diyetisyenliği Günleri'nde sözel bildiri olarak sunulmuştur. 21-22.02.2019, Adana

Author Contributions: Concept/Design : OC, İK; Data acquisition: OC; Data analysis and interpretation: OC; Drafting manuscript: OC, İK; Critical revision of manuscript: OC, İK; Final approval and accountability: OC, İK; Technical or material support: IK; Supervision: OC; Securing funding (if available): n/a.
Ethical Approval: The study was approved by our local ethics committee (Erzurum Regional Training and Research Hospital 2017 / 37732058-514.10).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support
Acknowledgement: This study is presented as an oral presentation at the XVII. Çukurova Pediatrics Symposium, Çukurova Pediatrics Nursing and Dietitian Symposium. (21-22.02.2019, Adana)

REFERENCES

1. Moch H, Humphrey PA, Ulbright TM, Reuter VE. Tumours of the urinary tract. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE, Editor. WHO Classification of Tumours of the Urinary System and Male Genital Organs, 4th ed. Lyon,France:IARC Press; 2016;77-106.
2. Arias-Stella JA, Shah AB, Gupta NS, Williamson SR. CK20 and p53 immunohistochemical staining patterns in urinary bladder specimens with equivocal atypia. Arch Pathol Lab Med. 2018;142:64-9.
3. Cheng L, Cheville JC, Neumann RM, Leibovich BC, Egan KS, Spotts BE et al. Survival of patients with carcinoma in situ of the urinary bladder. Cancer. 1999;85:2469-74.
4. Ogata DC, Marcondes CA, Tuon FF, Busato WF Jr, Cavalli G, Czeczko LE. Superficial papillary urothelial neoplasms of the bladder (PTA E PT1): correlation of expression of P53, KI-67 and CK20 with histologic grade, recurrence and tumor progression. Rev Col Bras Cir. 2012;39:394-400.
5. Wang G, McKenney JK. Urinary Bladder Pathology: World Health Organization Classification and American Joint Committee on Cancer Staging Update. Arch Pathol Lab Med. 2019;143:571-7.
6. Mallofr´e C, Castillo M, Morente V, Sol ´e M. Immunohistochemical expression of CK20, p53, and Ki-67 as objective markers of urothelial dysplasia. Mod Pathol. 2003;16:187–91.
7. Alsheikh A, Mohamedali Z, Jones E, Masterson J, Gilks CB. Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors. World/Health Organization /Internattional Society of Urologic Pathology. Mod Pathol. 2001;14:267-72.
8. Moll R. Molecular diversity of cytokeratin: significance for cell and tumor differentiation. Acta Histochem Suppl. 1991;4:117–27.
9. Harnden P, Eardley I, Joyce AD, Southgate J. Cytokeratin 20 as an objective marker of urothelial dysplasia. Br J Urol. 1996;78:870–5.
10. Rajcani J, Kajo K, Adamkov M, Moravekova E, Lauko L, Felcanova D et al. Immunohistochemical characterization of urothelial carcinoma. Bratisl Lek Listy. 2013;114: 431-8.
11. Ramos D, Navarro S, Villamón R, Gil-Salom M, Llombart-Bosch A. Cytokeratin expression patterns in low-grade papillary urothelial neoplasms of the urinary bladder. Cancer. 2003;97:1876-83.
12. Kim JK, Moon KC, Jeong CW, Kwak C, Kim HH, Ku JH. Papillary Urothelial neoplasm of low malignant potential (PUNLMP) after initial TUR-BT: Comparative analyses with noninvasive low-grade papillary urothelial carcinoma (LGPUC). J Cancer. 2017;8:2885-91.
13. Miyamoto H, Miller JS, Fajardo DA, Lee TK, Netto GJ, Epstein JI. Non-invasive papillary urothelial neoplasms: the 2004 WHO/ISUP classification system. Pathol Int. 2010;60:1-8.
14. Pan CC, Chang YH, Chen KK, Yu HJ, Sun CH, Ho DM. Prognostic significance of the 2004 WHO/ISUP classification for prediction of recurrence, progression, and cancer-specific mortality of non-muscle-invasive urothelial tumors of the urinary

bladder: a clinicopathologic study of 1,515 cases. Am J Clin Pathol. 2010;133:788-95.
15. Alrashidy M, Atef A, Baky TA. Immunohistochemical differentiation between urothelial papillomas and papillary neoplasms of low malignant potential of the urinary bladder. Asian Pac J Cancer Prev. 2016;17:1769-72.
16. Van Oers JM, Wild PJ, Burger M, Denzinger S, Stoehr R, Rosskopf E. FGFR3 mutations and a normal CK20 staining pattern define low-grade non-invasive urothelial bladder tumours. Eur Urol. 2007;52:760-8.
17. Mumtaz S, Hashmi AA, Hasan SH, Edhi MM, Khan M. Diagnostic utility of p53 and CK20 immunohistochemical expression grading urothelial malignancies. Int Arch Med. 2014;7:36.
18. Bertz S, Otto W, Denzinger S, Wieland WF, Burger M, Stöhr R et al. Combination of CK20 and Ki-67 immunostaining analysis predicts recurrence, progression, and cancer-specific survival in pT1 urothelial bladder cancer. Eur Urol. 2014;65:218-26.
19. Abdul-Maksoud RS, Shalaby SM, Elsayed WS, Elkady S. Fibroblast growth factor receptor 1 and cytokeratin 20 expressions and their relation to prognostic variables in bladder cancer. Gene. 2016;591:320-6.
20. Sikic D, Keck B, Wach S, Taubert H, Wullich B, Goebell PJ et al. Immunohistochemical subtyping using CK20 and CK5 can identify urothelial carcinomas of the upper urinary tract with a poor prognosis. PLoS One. 2017;12:e0179602.