ვადაზე სტატისტიკურად სარწმუნოდ ($p<0,05$) განსხვავდება ერთმანეთისგან. თუმცა, ერთნაირი ფოროვანობის მქონე ნიმუშების გამძლეობის ზღვრის საშუალო მაჩვენებლებს, მათი ჰიდრატაციის ვადისგან დამოკიდებულებით, აქვს ტენდენცია შემცირებისაკენ, მაგრამ ეს ცვლილებები დაკვირვების უკიდურეს ვადებზედაც კი არ აღწევს სტატისტიკურ სარწმუნოობას, რაც დასტურდება სტატისტიკური p მაჩვენებლის მნიშვნელობებით - 0,07; 0,759 და 0,124 ფოროვანობის 40%-, 30%- და 20%-ის ნიმუშებში, შესაბამისად.

პოლილაქტიდის და ტრიკალციფოსფატის ბაზაზე 3D-ბეჭდვის ტექნოლოგიით დამზადებული მასალის ნიმუშების გამძლეობის ზღვარი დამოკიდებულია მათ ფოროვანობაზე: რაც ნაკლებია ფორების მოცულობა, მით გამძლეა ნიმუშები. ნიმუშების ფიზიოლოგიურ ხსნარში 20 დღის განმავლობაში ჰიდრატაცია, ფორების მოცულობისაგან დამოუკიდებლად, არ იწვევს გამძლეობის სტატისტიკურად სარწმუნო ცვლილებებს, თუმცა ყველა გამოკვლეულ ნიმუშში აღინიშნება გამძლეობის ზღვრის საშუალო მნიშვნელობების შემცირების ტენდენცია.

## MOLECULAR CHARACTERISTICS OF THE HETEROGENEITY OF NON-INVASIVE PAPILLARY UROTHELIAL CARCINOMAS AND THE MARKERS OF THEIR RECURRENCE

**Kajaia D., Kochiashvili D., Muzashvili T., Gachechiladze M., Burkadze G.**

*Tbilisi State Medical University, Georgia*

Urothelial carcinoma represents the most common type of bladder cancer (>90%) and is the most frequent malignancy of the urinary tract [1]. It is the ninth most common cancer around the world, with the greater incidence in men [1]. Most of the cases of urothelial carcinoma are non-muscle invasive at the time of diagnosis [2]. However, in almost 70% of cases the recurrence is developed within 5 years of operation and 10-20% of them is present with more advanced, metastatic disease [3]. Therefore, under the similar pathologic staging and grading, the recurrence and progression of urothelial carcinoma vary significantly among individuals, which is attributed to tumor heterogeneity [4].

There are four major types of tumor heterogeneity, including (1) molecular and cellular differences between the same tumors in different patients (intertumoral heterogeneity), (2) differences in cancer cell types and/or molecular attributes within one tumor in a single patient (intratumoral heterogeneity), (3) differences in between primary and metastatic lesions and/or two metastatic lesions in one patient (intermetastatic heterogeneity), (4) differences in cancer cell types and/or molecular attributes in single metastatic lesion (intrametastatic heterogeneity) [5]. There are many different levels of tumor heterogeneity in each subtype and includes heterogeneity at the tissue, cellular and molecular level [5]. Frequently the morphologic heterogeneity of urothelial carcinomas, reflect their molecular heterogeneity [6]. Papillary urothelial cancers represent the heterogeneous group of lesions, with three major entities, including papillary urothelial neoplasms with low malignant potential (PUNLMP), low grade papillary urothelial carcinomas (LGPUC) and high grade papillary urothelial neoplasms (HGPUC) [7,8]. First one is characterised with relatively low recurrence rate [9]. However, due to higher tumor heterogeneity predicting the recurrence in patients with papillary bladder carcinoma is extremely difficult and currently there are lots of studies ongoing, which are investigating an additional morphometric, histopathological and immunohistochemical characteristics of papillary urothelial neoplasms.

Sangwan et al. [10], previously showed that mean nuclear area (MNA) measured by image analysis, as well as high proliferation index, measured as Ki67 labelling index represent two independent prognostic factors in patients with papillary urothelial neoplasms [10]. Akkalp et al., investigated the prognostic value of the presence of mitosis in haematoxylin and eosin stained specimens. They found that recurrent cases were characterised with the presence of ≥5 mitosis per HPF [11]. In addition, several studies identified the differential expression of cytokeratins to be predictive of the recurrence of non-invasive bladder carcinomas. For example, Jung et al., found that the loss of CK5/6 represents an independent prognostic factor for disease recurrence [12]. Jiang et al., also demonstrated the variable staining pattern of CK20 and CK7, which was corresponding the expression pattern in matched lymph node metastasis [13].

The aim of our study was to investigate the morphometric, histopathological and immunohistochemical characteristics of non-invasive papillary urothelial neoplasms, low and high grade papillary urothelial carcinomas, including nuclear area, stromal/parenchymal index, mitotic counts as defined by H&E and PHH3 staining, as well as proliferation activity, based on Ki67 labelling index and tumor tissue heterogeneity, based on the staining of cytokeratin 5, 7 and 20 (CK5, CK7, CK20).

**Material and methods.** Formalin fixed and paraffin embedded tissue material was retrieved from the Research, Diagnostic and Teaching Laboratory of Tbilisi State Medical University, Georgia. Study included altogether 81 tissue samples, divided into two following histopathological groups: normal urothelial epithelium (n=10), urothelial papilloma (n=15), urothelial neoplasms with low malignant potential (PUNLMP) (n=8), non-invasive low grade papillary urothelial carcinomas (LGPUC) (n=29) and non-invasive high grade papillary urothelial carcinomas (HGPUC) (n=19). In addition to basic study cohort, we have analysed 12 cases of relapsed papillary urothelial carcinomas (6 LGPUC and 6 HGPUC).

Standard haematoxylin and eosin stained specimens, were evaluated for the following nuclear features: nuclear area, nuclear perimeter and nuclear circularity using digital image analysis software QuPath. QuPath employs a machine learning approach, for significantly distinguishing various morphometric features.

The following algorithm of morphometric evaluation has been used: 10 HPF images were taken from each case and included in the program. After adjustment the staining vectors, the cell detection classifier has been applied which is based on the recognition of cell nuclei. Detected cells were analysed for the major nuclear features, including nuclear area, nuclear perimeter and nuclear circularity. Detection measurements were visualised as tables and histograms. In addition, the nuclear polymorphism has been also calculated, based on the variability of the nuclear area, perimeter and circularity. the presence of mitosis was counted manually, in the same images in QuPath software.

Tissue sections were stained using standard immunohistochemical procedure. Ready to use antibodies against the following antigens were used: Ki67 (K2), CK5(XM26), CK7 (RN7), CK20 (Ks20.8) (Leica). Staining and visualisation has been performed using Bond polymer refine detection system. The expression of all markers was evaluated as the percentage of marker positive cells, using digital analysis software QuPath. Following steps of image analysis has been applied: 10 HPF images were taken from each case, for each marker and included in the program. staining vectors were adjusted and the positive cell detection classifier was run. Detected positive and negative cells were transformed as detection points and recorded. In addition, for the evaluation of staining and therefore tumor phenotypic heterogeneity the results were visualised as histogram. In addition, the tumor tissue phenotypic heterogeneity was calculated as following: First, the intensity of the staining was evaluated as negative (0), weak (1+), moderate (2+) and strong (3+) by eye together with the percentage of each intensity for each tissue section and cases were classified as following: low heterogeneity – containing one of each above mentioned intensity in >50% of cells and high heterogeneity – containing ≥2 intensities in <50% of cells. Mitotic counts were obtained manually in the same software.

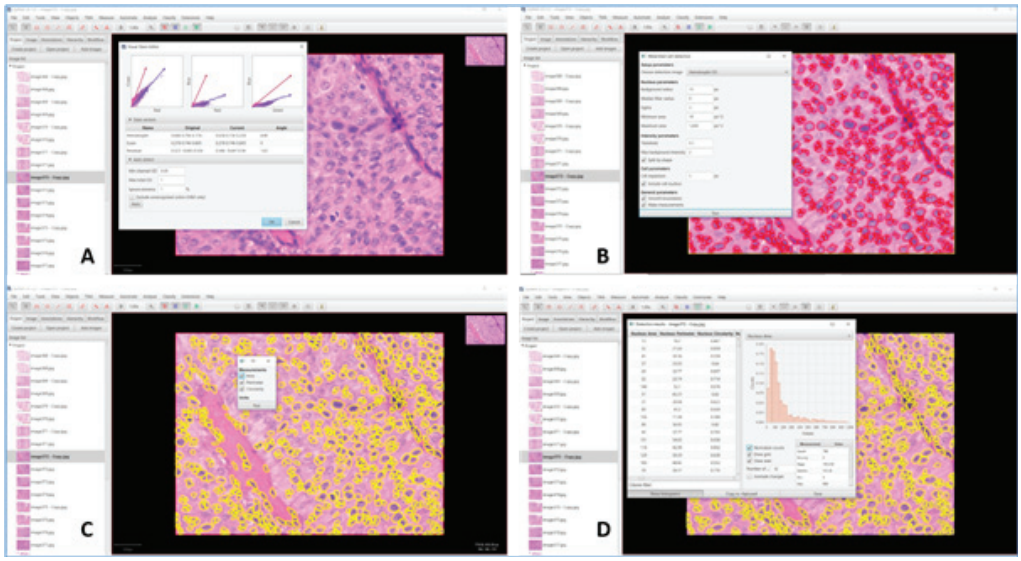



*Fig. 1. The morphometric evaluation algorithm in QuPath: A. adjustments of staining vectors, B. cell detection, C. nuclear feature analysis, D. visualisation of the results*

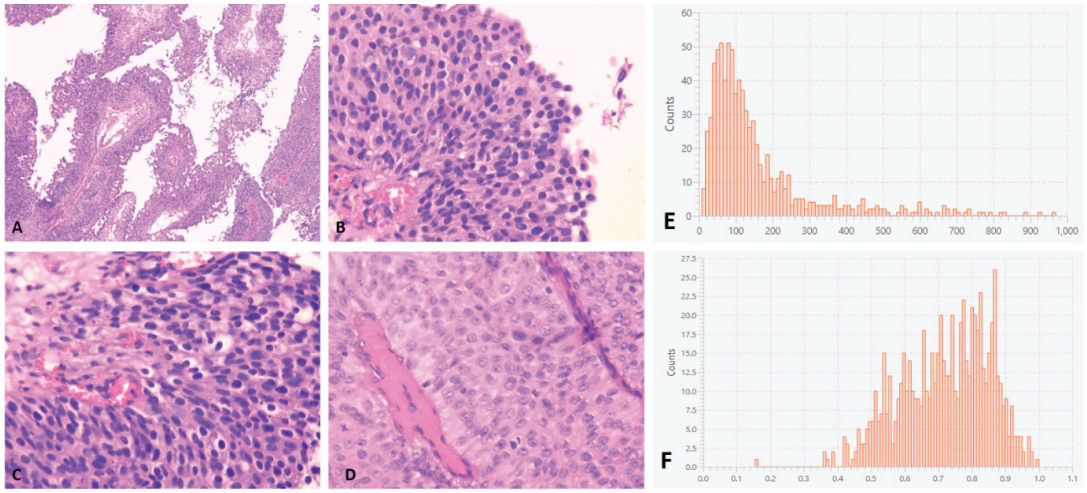



*Fig. 2. A. urothelial papilloma, H&E, x100, B. urothelial neoplasm with low malignant potential (PUNLMP), H&E, x400, C. non-invasive low grade papillary urothelial carcinoma (LGPUC), H&E, x400, D. non-invasive high grade papillary urothelial carcinoma (HGPUC), H&E, x400, E. non-invasive LGPUC showing relatively homogenous distribution of nuclear features, reflecting relatively lower level of nuclear polymorphism, F. non-invasive HGPUC showing relatively heterogeneous distribution of nuclear features, reflecting relatively higher level of nuclear polymorphism*

Comparisons between groups were made using Mann-Whitney U test and Kruskal-Wallis test. The Kruskal-Wallis test is a nonparametric (distribution free) test, and is used when the assumptions of one-way ANOVA are not met. The Kruskal-Wallis test can be used for both continuous and ordinal-level dependent variables. Correlations were assessed using Spearman's rank correlation. The Spearman's rank correlation is also used when data is non-parametrically distributed. P values <0.05 were considered as significant. All statistical tests were performed using SPSS software V20.00.

**Results and discussion.** Study of the nuclear feature distribution showed the following results: in normal urothelium the mean nuclear area was 56±20, in urothelial papilloma mean nuclear area was 102±45, in PUNLMP the mean nuclear area was 155±63, in non-invasive LGPUC the mean nuclear area was 241±96 and in non-invasive HGPUC mean nuclear area was 269±102. In normal urothelium, the mean nuclear perimeter was 20±6, in urothelial papilloma the mean nuclear perimeter was 42±12, in PUNLMP the mean nuclear perimeter was 51±21, in non-invasive LGPUC the mean nuclear area was 62±33 and in non-invasive HGPUC the mean nuclear perimeter was 64±35. Nuclear circularity in normal urothelium and in urothelial papilloma was 0.9±0.1, in PUNLMP it was 0.8±0.2, in non-invasive LGPUC nuclear circularity was 0.7±0.3 and in non-invasive HGPUC it was 0.6±0.4.

The study of the mitotic count evaluated in standard H&E stained sections did not detect any mitosis in normal urothelium and in urothelial papilloma, in PUNLMP the average mitotic count was 1.5±0.5, in non-invasive LGPUC it was 3.5±0.2 and in non-invasive HGPUC it was 12±1.5. Mitotic count evaluated as the PHH3 positive cell count also did not show the presence of any mitosis in normal urothelium and in urothelial papilloma. IN PUNLMP, the average number of PHH3 positive cells was 1.7±0.3, in non-invasive LGPUC it was 4.1±1.1 and in non-invasive HGPUC it was 11.6±2.

The study of the proliferation activity based on Ki67 labelling index showed the following results: in normal urothelium the immunohistochemical expression of Ki67 was not detected. In urothelial papilloma the mean Ki67 labelling index was 0.7±0.2, in PUNLMP the mean Ki67 labelling index was 2.9±0.8, in non-invasive LGPUC the mean Ki67 labelling index was 5.5±1.3 and in non-invasive HGPUC the mean Ki67 labelling index was 15.2±3.2.

The study of different cytokeratin distribution in groups showed the following results: the mean CK5 positivity was 17±2.9 in normal urothelium, 23±3.1 in urothelial papilloma, 27±3.3 in PUNLMP, 52±5.6 in non-invasive LGPUC and 59±7.8 in non-invasive HGPUC. The mean positivity for CK7 was 95±4.2 in normal urothelium, 74±6.8 in urothelial papilloma, 63±5.7 in PUNLMP, 47±3.6 in non-invasive LGPUC and 59±7.8 in non-invasive HGPUC. The mean positivity for CK20 was 15±4.8 in normal urothelium, 21±6.2 in urothelial papilloma, 24±7.7 in PUNLMP, 48±9.3 in non-invasive LGPUC and 54±10.2 in non-invasive HGPUC.

The analysis of the nuclear features in study groups indicated that the mean nuclear area is significantly increased in PUNLMP, non-invasive LGPUC and non-invasive HGPUC compared to normal urothelium and urothelial papilloma, reaching its maximum in HGPUC, similar to nuclear perimeter, which is also characterised with the similar distribution pattern.

The analysis of nuclear circularity showed that the maximum circularity is detected in normal urothelium and urothelial papillomas. Whilst in PUNLMP the nuclear circularity is slightly decreased. In non-invasive LGPUC and non-invasive HGPUC the level of nuclear circularity is significantly decreased. In addition, mentioned nuclear features were characterised with significant heterogeneous distribution in non-invasive LGPUC and HGPUC.

With regard to mitotic count, there were no mitosis detected in normal urothelium and urothelial papilloma, neither by evaluation of H&E stained tissues, nor by the evaluation of PHH3 immunohistochemistry. Mitotic count was generally lower in all PUNLMP cases, whilst it was significantly increased in non-invasive LGPUC and non-invasive HGPUC. Mitotic count was also characterised with significant heterogeneity in non-invasive LGPUC and HGPUC.

*Table 1. Distribution of nuclear characteristics and mitotic count in groups*

| | **Nuclear area** | **Nuclear perimeter** | **Nuclear circularity** | **Mitotic count/10HPF** | **PHH3** |
|---|---|---|---|---|---|
| Normal urothelium | 56±20 | 20±6 | 0.9±0.1 | 0 | 0 |
| Urothelial papilloma | 102±45 | 42±12 | 0.9±0.1 | 0 | 0 |
| PUNLMP | 155±63 | 51±21 | 0.8±0.2 | 1.5±0.5 | 1.7±0.3 |
| Non-invasive LGPUC | 241±96 | 62±33 | 0.7±0.3 | 3.5±0.2 | 4.1±1.1 |
| Non-invasive HGPUC | 269±102 | 64±35 | 0.6±0.4 | 12±1.5 | 11.6±2 |

*Table 2. Distribution of Ki67, CK5, CK7 and CK20 in study groups*

| | **Ki67** | **CK5** | **CK7** | **CK20** |
|---|---|---|---|---|
| Normal urothelium | 0 | 17±2.9 | 95±4.2 | 15±4.8 |
| Urothelial papilloma | 0.7±0.2 | 23±3.1 | 74±6.8 | 21±6.2 |
| PUNLMP | 2.9±0.8 | 27±3.3 | 63±5.7 | 24±7.7 |
| Non-invasive LGPUC | 5.5±1.3 | 52±5.6 | 47±3.6 | 48±9.3 |
| Non-invasive HGPUC | 15.2±3.2 | 59±7.8 | 36±2.2 | 54±10.2 |

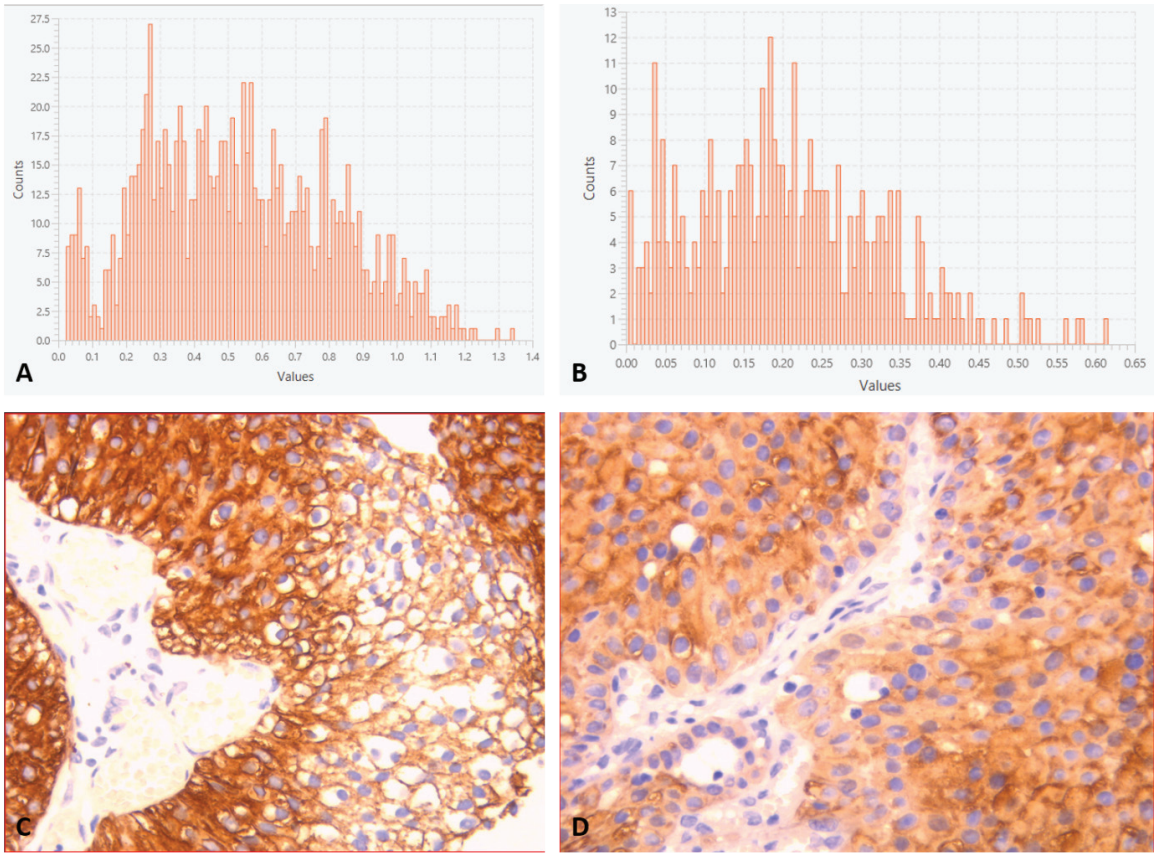
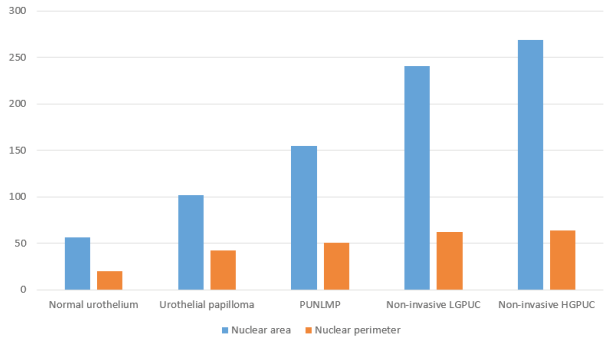
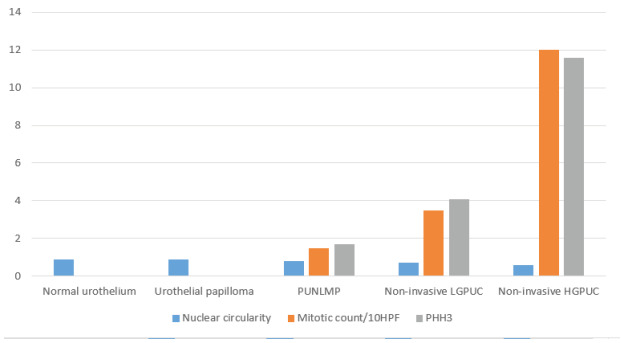


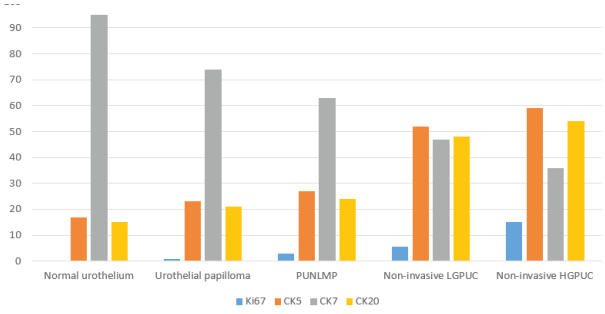

*Fig. 3. A. urothelial neoplasm with low malignant potential (PUNLMP) showing moderate heterogeneity in CK5 staining, B. non-invasive low grade papillary urothelial carcinoma (LGPUC) showing high grade heterogeneity of CK5 staining C - CK5 expression in urothelial neoplasm with low malignant potential (PUNLMP) IHC, x40 D - CK5 expression in non-invasive low grade urothelial carcinoma (LGPUC) IHC, x40*



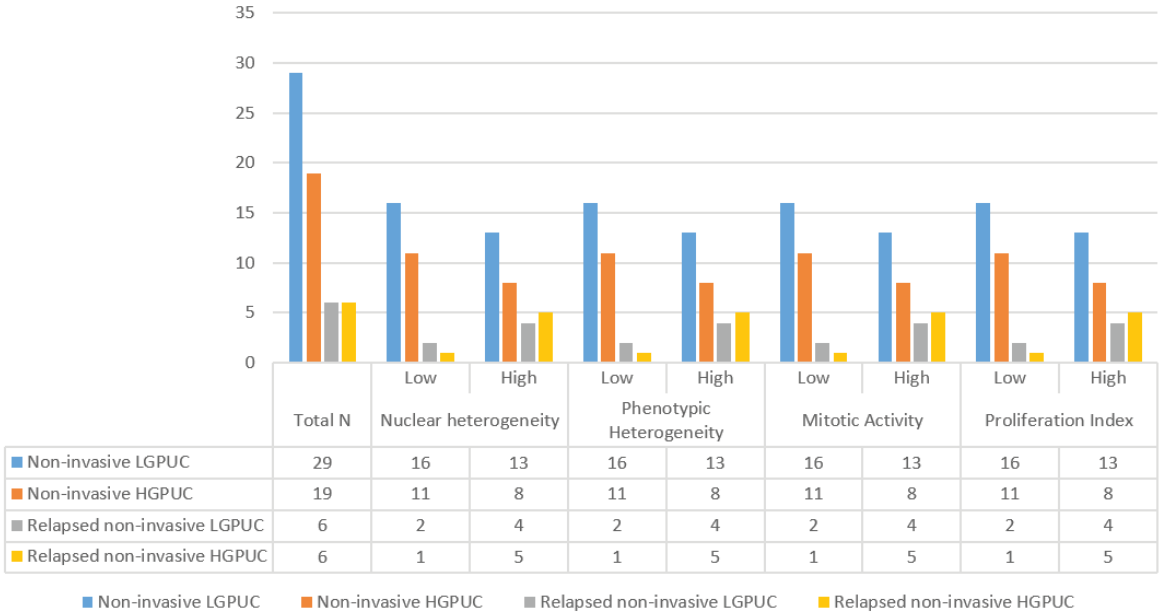

*Graph 1. Distribution of nuclear area and nuclear perimeter in study groups*




*Graph 2. Distribution of nuclear circularity and mitotic count in study groups*




*Graph 3. The distribution of Ki67, CK5, CK7 and CK20 in study groups*




| | Total N | Nuclear heterogeneity | | Phenotypic Heterogeneity | | Mitotic Activity | | Proliferation Index | |
|---|---|---|---|---|---|---|---|---|---|
| | | Low | High | Low | High | Low | High | Low | High |
| Non-invasive LGPUC | 29 | 16 | 13 | 16 | 13 | 16 | 13 | 16 | 13 |
| Non-invasive HGPUC | 19 | 11 | 8 | 11 | 8 | 11 | 8 | 11 | 8 |
| Relapsed non-invasive LGPUC | 6 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 |
| Relapsed non-invasive HGPUC | 6 | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 5 |

*Graph 4. Distribution of non-invasive LGPUC, HGPUC and relapsed cases in low and high risk groups*

Ki67 proliferation marker was not detected in normal urothelium and urothelial papilloma. In PUNLMP the proliferation activity was generally lower. The Ki67 proliferation index was also significantly increased in non-invasive LGPUC and HGPUC, showing the maximal labelling index in HGPUC. In addition, KI67 labelling index was characterised with maximal heterogeneity in non-invasive LGPUC and HGPUC groups.

The analysis of the distribution of cytokeratin markers in groups showed quite heterogenous results. In particular, CK5 expression was significantly increased from normal urothelium and urothelial papilloma, through PUNLMP and non-invasive LGPUC, reaching its maximum in non-invasive HGPUC. The expression of CK7 on the opposite was significantly decreased from normal urothelium to non-invasive HGPUC, whilst CK20 was significantly increased. In addition, the mentioned cytokeratins were characterised with the homogenous distribution pattern in normal urothelium, urothelial papilloma and PUNLMP, whilst they were characterised with highly heterogeneous distribution patterns in non-invasive LGPUC and HGPUC.

Providing the heterogeneity of the studied features in non-invasive LGPUC and HGPUCs, we have further performed the detailed analysis of the nuclear features and markers in this groups. In addition, we have investigated the mentioned characteristics in relapsed cases of non-invasive LGPUC and HGPUC. Analysis results showed that, the mentioned groups were divided into two major groups, particularly phenotype I was characterised with high heterogeneity based on nuclear features and cytokeratin expression, higher mitotic count and higher Ki67 labelling index, whilst phenotype II was characterised with relatively low heterogeneity, lower mitotic count and lower Ki67 labelling index.

In non-invasive LGPUC phenotype I was detected in 13/29 (44.8%) cases and in non-invasive HGPUC the phenotype I was detected in 11/19 (57.9%) cases. In relapsed cases, the phenotype I was detected in 4/6 (66.7%) cases in non-invasive LGPUC and 5/6 (83.3%) cases in non-invasive HGPUC. Based on this results, we may speculate that phenotype I tumors are characterised with the high risk of the development of subsequent relapse, compared to phenotypic II tumors.

One previous study also examined the morphometric characteristics of different urothelial lesions and similar to our study, they have also found that nuclear area is significantly increased in cancerous compared to non-cancerous tissues [10]. However, to the best of our knowledge we are first who also investigated nuclear perimeter and nuclear circularity features by morphometry in bladder lesions. We have found that in addition to nuclear area, the other morphometric characteristics, such as nuclear perimeter and nuclear circularity can be used for the distinguishing urothelial papilloma and PUNLMP, from the non-invasive LGPUC and HGPUC. In addition, providing the high heterogeneity of nuclear features in non-invasive LGPUC and HGPUCs, they can also be used to distinguish the groups, which are characterised with high risk of relapse.

Ko at al., also investigated the significance of Ki67 labelling index in non-muscle invasive bladder cancers. According to the results of the study of Ko et al., it has been shown that high level of Ki67 labelling index is associated with the increased risk of relapse [14]. In our study we have also found that phenotype I tumors which are characterised with high level expression on Ki67 are resembling to the relapsed cases, in which majority of them are also characterised with the higher expression of Ki67.

**Conclusions.** Nuclear features, as well as the number of mitosis, Ki67 labelling index and intratumoral heterogeneity significantly correlate with the presence of higher grade non-invasive urothelial lesions. In addition, it is possible to distinguish two major groups of non-invasive LGPUC and HGPUC, based on nuclear and phenotypic heterogeneity and mitotic count and Ki67 labelling index. I group which is characterised with higher intratumoral heterogeneity, higher mitotic count and higher Ki67 labelling index, represents the high risk group of non-invasive LGPUC and HGPUC recurrence.

## REFERENCES

1. Siegel R. L., Miller K. D. // Cancer Statistics , 2020," vol. 70, no. 1, pp. 7–30, 2020.
2. Cassell A. et al., Non-Muscle Invasive Bladder Cancer: A Review of the Current Trend in Africa. // World J. Oncol., vol. 10,

no. 3, pp. 123–131, Jun. 2019.
3. Bjurlin M. A. Management of Recurrent Non-Muscle Invasive Bladder Cancer. // Rev. Urol., vol. 20, no. 1, pp. 38–40, 2018.
4. Matulewicz R. S., Steinberg G. D. Non-muscle-invasive Bladder Cancer: Overview and Contemporary Treatment Landscape of Neoadjuvant Chemoablative Therapies. // Rev. Urol., vol. 22, no. 2, pp. 43–51, 2020.
5. Welch D. R. Tumor Heterogeneity--A 'Contemporary Concept' Founded on Historical Insights and Predictions. // Cancer Res., vol. 76, no. 1, pp. 4–6, Jan. 2016.
6. C. Chen et al. Bladder Tumor Heterogeneity: The Impact on Clinical Treatment // Urol. Int., vol. 95, no. 1, pp. 1–8, 2015.
7. Montironi R., Lopez-Beltran A., Mazzucchelli R. , Bostwick D. G.Classification and grading of the non-invasive urothelial neoplasms: recent advances and controversies // J. Clin. Pathol., vol. 56, no. 2, pp. 91 LP – 95, Feb. 2003.
8. Jones T. D., Cheng L. Reappraisal of the papillary urothelial neoplasm of low malignant potential (PUNLMP) // Histopathology, vol. 77, no. 4, pp. 525–535, Oct. 2020.
9. Rouprêt M, Malmstrom PU, Black P. Recurrent Ta Low-grade Non-muscle-invasive Bladder Cancer: What Are the Options? // Eur Urol Oncol. 2019 Nov;2(6):723-729. doi: 10.1016/j.euo.2019.06.003. Epub 2019 Jun 22.
10. Sangwan M, Singh S, Kumar S, Chabbra S, Sen R, Rana P, Malik S, Singh S, Lamba R. Role of morphometry and proliferative parameters in grading of urothelial neoplasms. // Cent European J Urol. 2015;68(1):37-44. doi: 10.5173/ceju.2015.01.447. Epub 2015 Mar 13.
11. Akkalp A. K., Onur Ö., Tetikkurt Ü. S., Tolga D., Özsoy S., Müslümanoğlu A. Y. Prognostic Significance of Mitotic Activity in Noninvasive, Low-Grade, Papillary Urothelial Carcinoma. // Anal. Quant. Cytopathol. Histopathol., vol. 38, no. 1, pp. 23–30, Feb. 2016.
12. Jung M., Kim B., Moon K. C. Immunohistochemistry of cytokeratin (CK) 5/6, CD44 and CK20 as prognostic biomarkers of non-muscle-invasive papillary upper tract urothelial carcinoma. // Histopathology, vol. 74, no. 3, pp. 483–493, Feb. 2019.
13. Jiang J. et al. Cytokeratin 7 and cytokeratin 20 in primary urinary bladder carcinoma and matched lymph node metastasis.// Arch. Pathol. Lab. Med., vol. 125, no. 7, pp. 921–923, Jul. 2001.
14. Ko K., Jeong C. W., Kwak C., Kim H. H., Ku J. H. Significance of Ki-67 in non-muscle invasive bladder cancer patients: a systematic review and meta-analysis.// Oncotarget 2017; vol. 8, no. 59, pp. 100614–100630.

## SUMMARY

### MOLECULAR CHARACTERISTICS OF THE HETEROGENEITY OF NON-INVASIVE PAPILLARY UROTHELIAL CARCINOMAS AND THE MARKERS OF THEIR RECURRENCE

**Kajaia D., Kochiashvili D., Muzashvili T., Gachechiladze M., Burkadze G.**

*Tbilisi State Medical University, Georgia*

Urothelial carcinoma represents the most common type of bladder cancer (>90%) and is the most frequent malignancy of the urinary tract. Most of the urothelial carcinomas are non-invasive at the time of diagnosis, however they are characterised with the risk of recurrence after surgical treatment. The aim of our study was to investigate the characteristics of tumor heterogeneity and markers of its progression in urothelial papillary carcinomas. Study included following groups: normal urothelial epithelium, urothelial papilloma, urothelial neoplasms with low malignant potential (PUNLM), non-invasive low grade papillary urothelial carcinomas (LGPUC) and non-invasive high grade papillary urothelial carcinomas (HGPUC). In addition, study included relapsed cases of non-invasive LGPUC and HGPUC. Nuclear features and mitotic counts was assessed using digital pathology software QuPath in standard H&E stained specimens. In addition, the presence of mitosis was detected as PHH3 labelled cells by immunohistochemistry. Proliferation was measured as Ki67 labelling index by immunohistochemistry. Tumor heterogeneity was investigated by the differential expression pattern of CK5, CK7 and CK20 by immunohistochemistry. Study results showed, that Nuclear features, as well as the number of mitosis, proliferation index and intratumoral heterogeneity significantly correlate with the presence of higher grade non-invasive urothelial lesions. In addition, it is possible to distinguish two major groups of non-invasive LGPUC and HGPUC, based on nuclear and phenotypic heterogeneity and mitotic and proliferative activity, I group which is characterised with higher intratumoral heterogeneity, higher mitotic count and higher proliferative activity, represents the high risk group of non-invasive LGPUC and HGPUC recurrence.

**Keywords:** Urothelial carcinoma, bladder cancer, normal urothelial epithelium, urothelial papilloma, urothelial neoplasms with low malignant potential (PUNLM), non-invasive low grade papillary urothelial carcinomas (LGPUC), non-invasive high grade papillary urothelial carcinomas (HGPUC).

## РЕЗЮМЕ

### МОЛЕКУЛЯРНЫЕ ХАРАКТЕРИСТИКИ ГЕТЕРОГЕННОСТИ И МАРКЕРЫ РЕЦИДИВА НЕИНВАЗИВНЫХ ПАПИЛЛЯРНЫХ КАРЦИНОМ МОЧЕВОГО ПУЗЫРЯ

**Каджая Д.Р., Кочиашвили Д.К., Музашвили Т.З., Гачечиладзе М.Д., Буркадзе Г.М.**

*Тбилисский государственный медицинский университет, Грузия*

Уротелиальная карцинома составляет >90% опухолей мочевого пузыря, также являясь самой распространенной патологией мочевыводящих путей. Большинство папиллярных карцином мочевого пузыря в момент обнаружения неинвазивны, однако характеризуются определённым риском развития рецидива.

Целью исследования явилось определение маркеров опухолевой гетерогенности и прогрессии в уротелиальных папиллярных карциномах.

Исследование состояло из следующих групп: нормальный уротелий, уротелиальная папиллома, уротелиальные неоплазии с низким потенциалом злокачественности (PUNLM), неинвазивная уротелиальная папиллярная карцинома низкой степени злокачественности (LGPUC) и неинвазивная уротелиальная папиллярная карцинома высокой степени злокачественности (HGPUC). Исследование также включало случаи рецидивов LGPUC и HGPUC. Ядерные показатели и митотическая активность изучены в препаратах, окрашенных стандартным гематоксилином и эозином

с использованием программы цифровой патологии QuPath. Для оценки митотической активности использовано антитело PHH3. Пролиферативная активность оценена Ki67 индексом, иммуногистохимическим методом. Опухолевая гетерогенность изучена маркерами CK5, CK7 и CK20 иммуногистохимическим методом.

Результаты исследования показали, что ядерные показатели, также как количество митозов, пролиферативный индекс и опухолевая гетерогенность достоверно ассоциируются с наличием высокой степени поражения. Возможно выделение двух групп с различным фенотипом в LGPUC и HGPUC, из которых первый фенотип характеризуется высокой интраопухолевой гетерогенностью, высокой митотической и пролиферативной активностью и представляет группу высокого риска развития рецидива LGPUC и HGPUC.

რეზიუმე

შარდის ბუშტის არაინვაზიური პაპილური კარცინომების ჰეტეროგენულობის მოლეკულური მახასიათებლები და რეციდივის მარკერები

დ.ქაჯაია, დ.ქოჩიაშვილი, თ. მუზაშვილი, მ. გაჩეჩილაძე, გ. ბურკაძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, საქართველო

უროთელური კარცინომა წარმოადგენს შარდის ბუშტის სიმსივნეების 90%-ზე მეტს. იგი ასევე არის საშარდე გზის ყველაზე გავრცელებული სიმსივნური პათოლოგია. შარდის ბუშტის პაპილური კარცინომების დიდი ნაწილი დადიაგნოზების დროს არაინვაზიურია, თუმცა ისინი ხასიათდებიან რეციდივის განვითარების გარკვეული რისკით.

კვლევის მიზანს შეადგენდა სიმსივნის ჰეტეროგენულობის და პროგრესიის მოლეკულური მარკერების შესწავლა უროთელურ პაპილურ კარცინომებში. კვლევა მოიცავდა შემდეგ საკვლევ ჯგუფებს: ნორმალურ უროთელიუმს, უროთელიურ პაპილომას, უროთელურ ნეოპლაზიებს დაბალი ავთვისებიანობის პოტენციალით (PUNLMP), არაინვაზიურ დაბალი ხარისხის ავთვისებიანობის უროთელურ პაპილურ კარცინომას (LGPUC) და არაინვაზიურ მაღალი ხარისხის ავთვისებიანობის პაპილურ უროთელურ კარცინომას (HGPUC). კვლევა მოიცავდა LGPUC და HGPUC-ის რეციდივულ შემთხვევებს. ბირთვული მახასიათებლები და მიტოზური აქტივობა შეფასებული იყო სტანდარტულ ჰემატოქსილინით და ეოზინით შეღებილ ანათლებში, ციფრული პათოლოგიის პროგრამის QuPath-ის გამოყენებით; გარდა ამისა, მიტოზური აქტივობა შეფასებული იქნა PHH3-ით. პროლიფერაციული აქტივობა შეფასებული იყო Ki67 მონიშვნის ინდექსით, იმუნოჰისტოქიმიური მეთოდით. სიმსივნის ჰეტეროგენულობა შეფასდა CK5, CK7 და CK20-ის იმუნოჰისტოქიმიური გამოკვლევით.

კვლევის შედეგებმა აჩვენა, რომ ბირთვული მახასიათებლები, ისევე როგორც მიტოზების რაოდენობა, პროლიფერაციული ინდექსი და სიმსივნის ჰეტეროგენულობა სარწმუნოდ ასოცირდება მაღალი ხარისხის დაზიანებების არსებობასთან. შესაძლებელია ორი ფენოტიპურად განსხვავებული ჯგუფის გარჩევა LGPUC და HGPUC-ში, რომელთაგან პირველი ფენოტიპი, რომელიც ხასიათდება მაღალი ინტრასიმსივნური ჰეტეროგენულობით, მაღალი მიტოზური და პროლიფერაციული აქტივობით, წარმოადგენს LGPUC და HGPUC რეციდივის განვითარების მაღალ რისკ-ჯგუფს.

* * *