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Interobserver agreement and the role of smoothelin immunostain in the evaluation of Muscularis Propria (MP) invasion of Bladder Urothelial Carcinoma (BUC)

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ABSTRACT

Background: Bladder Urothelial Carcinoma (BUC) is common cancer in the genitourinary organs. One important determinant for its therapy modality is the presence of tumor invasion into Muscularis Propria (MP). Assessing MP invasion in routine Hematoxylin-Eosin (HE) slides of Transurethral Resection of Bladder (TUR-B) specimens is not always easy due to tissue fragmentation and morphological similarities of MP to muscularis mucosa (MM) and myofibroblasts. This study was done to determine the interobserver agreement in evaluating MP invasion and to find alternatives to overcome this diagnostic problem.

Methods: This cross-sectional observational analytic study was conducted in the Pathology Laboratory of the Faculty of Medicine, Universitas Udayana/Prof. Dr. I.G.N.G. Ngoerah Hospital, Bali, in 2022. As many as 72 HE slides of TUR-B specimens of BUC patients were examined separately by five experienced pathologists. Smoothelin immunostaining, a marker to identify MP, was also examined as a comparison. Data were processed and statistically tested using the SPSS version 20.0 program for Windows.

Results: The interobserver agreement of those five pathologists was found to be low to moderate, with an average κ = 0.48, ranging between 0.18-0.69. One pathologist had a low agreement with four others (κ<0.4) and indicated diagnostic uncertainty in 41% of the samples examined. Among four other pathologists, the agreements were moderate, with κ = 0.40-0.69. The proportion of concordant examination results was only 45.8%. When the outcome of each HE slide was concluded by consensus, it was found that there was no statistically significant difference with smoothelin immunostaining results.

Conclusion: Evaluation of MP invasion of BUC in TUR-B specimens had low to moderate interobserver agreement, which indicated that this examination was subjective. However, the consensus of several pathologists seems to lead to better accuracy, close to the result of smoothelin immunostaining.

Keywords: BUC, Interobserver Agreement, MP Invasion, Smoothelin Immunostaining.
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INTRODUCTION

Cancer is an important health problem due to its high morbidity and mortality rates, high cost of treatment, and psychological stress effect on the sufferers and their families. Cancer may arise in almost all body organs, including the bladder.[1] Bladder urothelial carcinoma (BUC) is common cancer in the genitourinary organs. In addition to genetic susceptibility, several environmental and behavioral factors were proven to contribute to the development of bladder cancer, such as certain dietary and herbal medicine exposures, radiation and medication, infections and chronic irritation, tobacco smoke, opium, and occupational exposure.[1]

Globocan 2020 data for Indonesia showed that bladder cancer was the 13th most common cancer, with 7,828 new cases or around 2% of the total cancer cases. The number of deaths from this cancer was 3,885 people, ranking the 14th highest of the total deaths from cancer, which was 1.7%.[2] During 2013-2017, as many as 127 bladder specimens were examined at the Pathology Laboratory of Sanglah Hospital (currently named Prof. Dr. I.G.N.G. Ngoerah Hospital), of which 70.9% were neoplastic diseases, and 58.9% of them were invasive BUC.[3]

The diagnosis of BUC is established based on histopathological examination of biopsy or Transurethral Resection of Bladder (TUR-B) specimens. BUC is divided into non-invasive, flat in-situ carcinoma or papillary, and invasive BUC based on the growth pattern. Determining the grade, histological subtypes, and depth of invasion of invasive BUC is mandatory. Those three histopathological parameters are important predictive factors for BUC

aggressiveness used by urologists to choose appropriate therapy modalities.[1,4]

This study concerns problematic issues in evaluating the depth of tumor invasion, particularly in assessing the presence of MP invasion. This evaluation aims to determine the invasive BUC's pathological tumor (pT) stage. The case without MP invasion is classified below the pT2 stage and grouped as Non-Muscle Invasive Bladder Cancer (NMIBC). In contrast, tumor with MP invasion is in the pT2 or above the stage and grouped into Muscle Invasive Bladder Cancer (MIBC). NMIBCs generally show remission after TUR-B with or without intravesical chemotherapy or immunotherapy, whereas MIBCs need more aggressive treatment, including cystectomy or/and systemic chemoradiation therapy.[4]

The accuracy of assessing the presence of MP invasion is crucial because any inaccurate decision will lead to under or over-treatment that is detrimental to the patients. Undertreatment will endanger because the patient received inadequate treatment, while over-treatment will reduce the patient's quality of life due to unnecessary cystectomy and chemotherapy. The problem is that assessing MP invasion in TUR-B specimens' Hematoxyllin-Eosin (HE) slide is not always easy. First, the tissue is fragmented into pieces of small tissue that are impossible to re-oriented, like a cystectomy specimen. Second, the possibility that the invaded part is not sampled, resulting in a false negative result. Third, morphological similarities of smooth muscle cells of the MP layer with the muscularis mucosa (MM) layer and reactive myofibroblast cells may lead to inaccurate diagnosis, particularly if they were invaded by massive tumor cells.[5]

Considering the high incidence of BUC cases and the importance of assessing MP invasion as the guide to therapy, this study was conducted. The aims were to determine the interobserver agreement in assessing MP invasion in HE slides among pathologists and to compare the results of HE slides interpretation with the smoothelin immunostaining results. In previous studies, smoothelin, a marker for differentiated smooth muscle cells, accurately distinguished MP, MM and myofibroblast. Smoothelin was moderate to strongly expressed in MP, not expressed or weakly expressed in MM, and not in myofibroblast cells.[6,7]

Based on those mentioned above, this study aims to provide information beneficial for patient care regulation, either at Prof. Dr. I G.N.G. Ngoerah Hospital or other hospitals with similar pathological resource characteristics, especially in terms of facilities and experiences of the pathologists.

METHODS

This study was an observational analytic study using a cross-sectional design. It was carried out at the Pathology Laboratory of Faculty of Medicine Universitas Udayana/ Prof. Dr. I G.N.G. Ngoerah Hospital, in 2022, after passing ethical eligibility from the health research ethics committee of Faculty of Medicine Universitas Udayana (Letter no. 1061/UN14.2.2.VII.14/ LT/2022) and obtaining a research permit letter from the director of Prof. Dr. I.G.N.G. Ngoerah hospital (Letter no.LB.02.01/XIV.2.2.2/25608/2022).

The subjects of this study were formalin-fixed paraffin-embedded (FFPE) tissue of TUR-B specimens from BUC patients who underwent histopathological examination at Prof. Dr. I.G.N.G. Ngoerah Hospital from January 2017 until May 2022. All subjects must meet inclusion and exclusion criteria. All FFPE tissue of TUR-B specimens from BUC patients still archived in the pathology laboratory were included in this study, regardless of type, subtype or grade. The exclusion criteria included FFPE tissue that was damaged or did not contain sufficient tumor mass for examination, recurrent cases, and patients with uncertain diagnoses. A total of 72 FFPE tissue were obtained by consecutive sampling.

From each FFPE tissue, two types of preparation were made, which were HE and smoothelin immunostaining. The standard routine procedure did him staining. The immunostaining was done by the indirect method using BOND-MAX Automated Immunohistochemistry Vision Biosystem (Leica Microsystems GmbH, Wetzlar, Germany) with primary smoothelin Clone R4A monoclonal antibody (BioSB).

Each HE slide was assessed by five pathologists separately. Their interpretation was grouped into five categories: (1) No visible MP, (2) Visible MP without tumor cell invasion, (3) uncertain MP invasion, which tends to be negative, (4) Uncertain MP invasion, which tends to be positive, and (5) Positive MP invasion. This categorization was based on the real results of compiling original data from the pathology answer sheet and was discussed with the five pathologists involved in this study. The agreement kappa was calculated using the SPSS version 20.0 program for Windows.

Furthermore, the interpretation of HE slides was grouped into two categories, positive and negative, based on the consensus of the five pathologists. Smoothelin immunostaining was performed following standard procedures, and the interpretation was made using strict criteria, hence reliably assessed by one pathologist. Bladder wall tissue from cystectomy was used as control tissue to guarantee proper and correct immune-staining procedure and to determine the level of color intensity. The results were classified into two categories: positive if the target cells had moderate to strongly expressed brown coloration in the cytoplasm and negative if the brown color was mild or none. The evaluation of HE and smoothelin was performed blindly. The association between HE and smoothelin interpretation was tested by the Chi-Square test using the SPSS version 20.0 program for Windows.

RESULTS

Clinicopathological profile of patients

A total number of 72 samples were obtained from 41 BUC patients by consecutive sampling. Patients who have more than 3 FFPE specimens (31 patients) were selected two times by randomization, while patients who have 1-3 FFPE specimens (10 patients) were selected one time by randomization; hence the total number of FFPE included in this study were 72.

The clinicopathological profile of the 41 patients is shown in Table 1. It can be seen that most cases of BUC were in the age group 70-79 years, followed by the age group 50-59 years. The number of

male patients was more predominant than female, with a ratio close to 4:1. About half of the cases were classic BUC (53.66%), and the rest were BUC subtypes, with the most common subtype being BUC with squamous differentiation. Two cases were BUC with divergent differentiation, consisting of squamous, glandular and small cell differentiation. About two-thirds of cases were high grade and the rest were low grade.

Table 1. Clinicopathological Profile of 41 BUC Patients in the Study

Characteristic	Number of Patients (n=41)	Percentage (%)
Age (year old)		
40-49	3	7.32
50-59	11	26.83
60-69	7	17.07
70-79	14	34.15
80-89	6	14.63
Sex		
Male	33	80.49
Female	8	19.51
Type/subtype		
Infiltrating (classic)	22	53.66
Subtype:		
BUC with squamous differentiation	9	21.94
BUC with glandular differentiation	3	7.32
BUC with divergent differentiation	2	4.88
Sarcomatoid carcinoma	2	4.88
Poorly differentiated carcinoma	1	2.44
Giant cell carcinoma	1	2.44
BUC with neuroendocrine-like feature	1	2.44
Grade		
Low grade	13	31.71
High grade	28	68.29

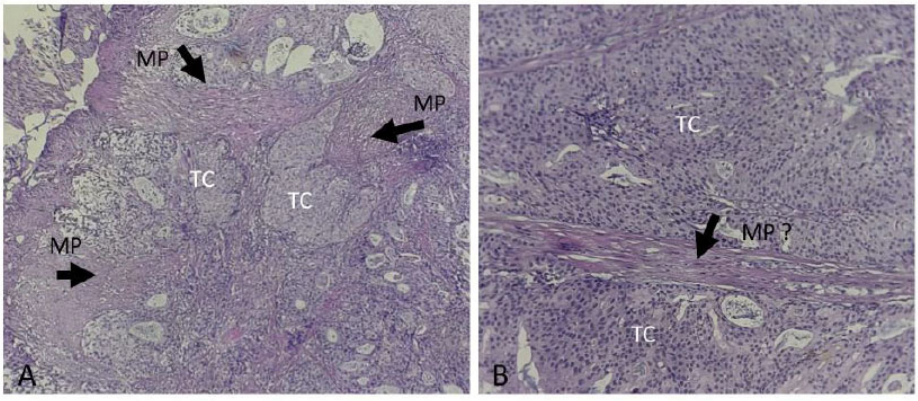

Figure 1. Microscopic pictures of BUC (HE, 100x). (A) The tumor cells (TC) are frankly invasive into the MP layer. (B) Massive tumor cells (TC) with a thin layer of muscle are seen in the central, uncertain whether it is MP, MM, or myofibroblast.

Results of MP Invasion Examination from HE slides

Both MP and MM were composed of smooth muscle cells with similar morphology, in which cells are spindle in shape, with cigar-shaped nuclei and eosinophilic cytoplasm. Figure 1 shows that the MP layer is not always as easily recognizable as in Figure 1A. When the invasive tumor is massive, only a small portion of muscle tissue is visible between the tumor cells, so there is doubt about whether there has been an MP invasion, as seen in Figure 1B.

Table 2 shows the result of the MP invasion evaluation by the five observers separately. Observers PA1, PA4 and PA5 showed a dominance of frankly positive results but with different frequencies, which were 34%, 37% and 29%, respectively. Observers PA2 and PA3 showed no MP tissue result dominance, with nearly the same frequency of 39% and 40%, respectively.

Table 3 focused on cases that were evaluated as uncertain categories. It is shown that there was a frank difference between observer PA4 and PA5, with the percentage of uncertain cases being 4% vs. 41%. Two other observers, PA1 and PA2, show the same percentage of uncertain cases (each 19%), while PA3 offers 29%. The mean of uncertain cases of the five observers was 22.4%.

Table 4 is the re-grouping form of Table 2. In Table 4, the result of the MP invasion evaluation was grouped into only two categories. Slides with frank MP invasion and uncertain but tend to be positive results were categorized as positive MP invasion. In contrast, slides with no

Table 2. The Results of MP Invasion Examination on HE Slides by Five Observers

Observers	MP invasion (n=72)				
	No MP n (%)	Invasion (-) n (%)	Uncertain, likely (-) n (%)	Uncertain, likely (+) n (%)	Invasion (+) n (%)
PA1	18 (25.0)	7 (10.0)	4 (6.0)	9 (13.0)	34 (47.0)
PA2	28 (39.0)	6 (8.0)	6 (8.0)	8 (11.0)	24 (33.0)
PA3	29 (40.0)	5 (7.0)	6 (8.0)	15 (21.0)	17 (24.0)
PA4	22 (31.0)	10 (14.0)	2 (3.0)	1 (1.0)	37 (51.0)
PA5	11 (15.0)	2 (3.0)	6 (8.0)	24 (33.0)	29 (40.0)

MP: Muscularis Propria

MP tissue, frankly no MP invasion and uncertain but tend to be negative results were grouped into negative MP invasion.

Table 4 shows that observers PA1, PA4 and PA5 got more positive than negative results, while PA2 and PA3 found more negative than positive results.

Table 3. The Distribution of Uncertain MP Invasion Cases by Five Observers

Observers	Percentage of uncertain result (N=72)		
	Uncertain, likely (-) n (%)	Uncertain, likely (+) n (%)	Total uncertain cases n (%)
PA1	4 (6.0)	9 (13.0)	13 (19.0)
PA2	6 (8.0)	8 (11.0)	14 (19.0)
PA3	6 (8.0)	15 (21.0)	21 (29.0)
PA4	2 (3.0)	1 (1.0)	3 (4.0)
PA5	6 (8.0)	24 (33.0)	30 (41.0)
x̄	4.8 (6.67)	37.8 (15.80)	16.2 (22.40)

Table 4. Distribution of Evaluation of MP Invasion in Two Categories

Observers	Result of MP invasion (n=72)	
	Negative n (%)	Positive n (%)
PA1	29 (40.0)	43 (60.0)
PA2	40 (56.0)	32 (44.0)
PA3	40 (56.0)	32 (44.0)
PA4	34 (47.0)	38 (53.0)
PA5	19 (26.0)	53 (74.0)

MP: Muscularis Propria

Table 5. Interobserver Agreement in Evaluation of MP Invasion

Observers	Kappa value (κ)	p
PA1.PA2	0.59	<0.001
PA1.PA3	0.43*	<0.001
PA1.PA4	0.69	<0.001
PA1.PA5	0.33*	0.004
PA2.PA3	0.66	<0.001
PA2.PA4	0.67	<0.001
PA2.PA5	0.29*	0.003
PA3.PA4	0.67	<0.001
PA3.PA5	0.18*	0.064
PA4.PA5	0.29*	0.007

*Kappa value less than 0.50

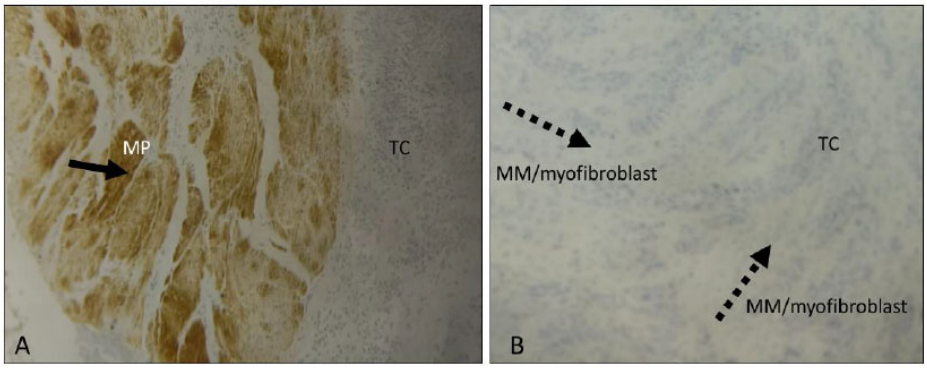

Figure 2. Microscopic pictures of Smoothelin immunostaining (100x magnification). A. Brown coloration with moderate-strong intensity on MP. B. No brown coloration is seen in the muscle spindle cell area between the tumor cells (TC), indicating that it is not MP.

Interobserver Agreement Among Five Pathologists in the Evaluation of MP Invasion

Table 5 shows the agreement between two of the total five observers. It shows that the interobserver agreements varied greatly. The highest agreement occurred between observers PA1 and PA4, with Ķ = 0.69. The lowest agreement occurred between observers PA3 and PA5, with Ķ = 0.18. Observer PA5 also showed low agreement with the other four observers, with Ķ < 0.4. Among the four other observers, the agreements were moderate.

In addition, although Table 4 shows that the PA2 and PA3 have the same number for positive and negative results, the agreement analysis between the two only shows Ķ = 0.66, as shown in Table 5.

Result of Smoothelin Immunostaining and Its Association with the Result of HE

Smoothelin immunostaining was performed in all 72 samples. The microscopic pictures are presented in Figure 2. The result was categorized into two categories, positive and negative. The distribution of smoothelin results is shown in Table 6, in association with HE results. The table shows that the smoothelin detected more positive samples than HE, 61.1% versus 52.8%. A total of 9 negative samples on HE was found positive on smoothelin. Conversely, as many as 3 positive samples for HE did not prove positive for smoothelin. However, the concordance between smoothelin and HE was quite high, where 35 (48.6%) samples were both rated positive, and as many as 25 (34.7%) samples were both negative. Only 12 out of 72 samples showed differences in results between consensus HE and smoothellin. Chi-square analysis showed that the association between the two tests was statistically significant, with a p<0.001 (Table 6).

DISCUSSION

BUC is divided into two categories based on the depth of its invasion, namely, Non-Muscle Invasive Bladder Cancer (NMIBC) and Muscle Invasive Bladder Cancer (MIBC). The two categories of BUC occur

Table 6. Results of Evaluation of MP Invasion on Smoothelin Immunostaining and HE

IHC Smoothelin	HE (n=72)		Total	p
	Positive n (%)	Negative n (%)		
Positive	35 (48.6)	9 (12.5)	44 (61.1)	<0.001*
Negative	3 (4.2)	25 (34.7)	28 (38.9)	
Total	38 (52.8)	34 (47.2)	72 (100.0)	

IHC: Immunohistochemistry; HE: Haemtoxylin Eosin; *Chi-Square: Statistically significant if p-value less than 0.05

through different carcinogenic pathways and show other biological traits, so they require additional treatments. Most MIBCs arise de novo, although about 10-20% are from NMIBC progression. The MIBC group is more aggressive with a poorer prognosis. Early-stage MIBC has a 5-YSR of 60%, while those with advanced stages <10%.[8]

The problem of the difficulty in evaluating MP muscle invasion in HE slides of TUR-B specimens has been recognized for a long time, and many studies have been reported on this issue. There are several reasons underlying this problem. First, in TUR-B, the tumor tissue is not removed in toto. The tumor was fragmented into many small size tissues, so re-orientation of bladder wall layers is impossible. Second, MP shares some similar morphology with MM and myofibroblasts. If the tumor cells are too massive to infiltrate the MP layer, the thin muscle bundles between massive tumor cells will be difficult to distinguish from MM and myofibroblasts. Conversely, in some cases, MM is found to have hyperplasia so that its thickness can be mistaken for MP.[5]

Previously, a retrospective study by Poletajew et al. in 2014 assessed the concordance between the BUC stages of TUR-B samples and radical cystectomy (RC). Both were evaluated from HE slides. A total of 181 patients were included in their study. The results showed poor agreement, in which only 31.6% of cases were found in concordant stages between TUR-B and RC. A total of 13.8% of cases were found to be at a lower stage and 54.6% of cases at a higher stage when evaluated from RC.[9]

Most pathology diagnostic centers in Indonesia, including Prof. Dr. I.G.N.G Ngoerah Hospital, used HE staining to evaluate MP invasion. To overcome the first problem above, standardized TUR-B specimen handling techniques are needed. All TUR-B tissue arriving at the pathology laboratory must be weighed and thoroughly processed, especially if the total tissue weight is 10 grams or less. If a papillary lesion can be identified, the number of tissue fragments containing the lesion and the macroscopic size of the tissue fragments should be recorded. Then, the tissue is processed in a cassette where every centimeter of the tumor is minimally processed in one cassette. Maximum specimens are processed in 10 cassettes. Specimens with a more than 10 grams volume require additional cassettes until proven that there is or is not an MP invasion. This method can reduce the possibility of false negative results due to unsampling.[10]

When TUR-B is performed, the European Association of Urology (EAU) recommends a different specimen handling technique by placing tumor sections in separate containers between the exophytic lesion, the tumor base and the resection margins. By this, it is hoped that the evaluation of the depth of invasion will be more accurate.[10]

In addition to improving specimen handling techniques, the selection of surgical procedures is also expected to increase the accuracy of assessing the depth of invasion. In developed countries with available facilities, tumors with a preoperative diagnosis of NMIBC and measuring 1 cm or less were operated on with the en-bloc technique. In this technique, a circumferential incision is made in the bladder mucosa at a distance of several millimeters from the margin of the tumor. With this technique, the tumor mass is received intact in the pathology lab, its orientation is maintained, and its depth of invasion can be properly assessed. Currently, this technique is being studied for use on larger tumors (maximum 3 cm).[10]

The second problem arises not only because of the observer's level of competence and experience. MP, MM and myofibroblast muscle cells indeed have morphological similarities. Each cell is spindle-shaped, with a cigar-shaped nucleus and eosinophilic cytoplasm. These three will be difficult to distinguish from TUR-B specimens because of their random position, massive infiltrative tumor cells into the MP, or hyperplastic MM or myofibroblasts. Differentiating the three is easier to do in RC specimens because the position of MP is more external than that of MM or myofibroblasts in the mucosa.[5,9]

In our study, the level of uncertain results in assessing MP invasion was quite high, with an average of 22.4%. One observer even shows uncertain results in 41% of samples. This is in line with the result of the interobserver agreement level. The average kappa value between the 2 observers is 0.58, ranging from 0.18-0.69. All observers evaluated the same slides, so sample bias did not affect the agreement. Differences in observer competence may have had an effect. Still, this study minimized it by involving five observers who worked in the same hospital for more than 5 years and had clinical privileges to evaluate TUR-B specimens.

One solution to overcome this second problem is to use a marker that can distinguish the three types of cells. Mahde & Al Asadi studied Smoothelin and Vimentin to differentiate MP and MM and found that 5 of 60 cases with HE diagnosed with pT1 were pT2 cases when evaluated with Smoothelin.[11] Previously, Elkady et al. also conducted studies with smoothelin and vimentin.[6] They used 59 RC specimens with BUC and 11 bladder biopsy specimens with a diagnosis of chronic inflammation. The results showed that the smoothelin expression intensity and positivity percentage were significantly higher in MP than in MM.

Vimentin was not stained at all MP and was stained at 80% MM. They concluded that the combination of positive smoothelin staining with medium-strong intensity and negative vimentin staining had 100% sensitivity and specificity in identifying MP.[6]

Another study by Mihai et al. found that the smoothelin expression intensity tended to be stronger in MP compared to MM and vascular muscle.[12] But, examination with smoothelin is recommended with caution because the staining technique also affects the results. From this study, it was found that 33.3% of BUC, whose staging was dubious in HE, could be staged with the help of Smoothelin.[12]

Apart from vimentin and smoothelin, several other markers have been tested by other researchers. Kinra et al. studied the expression of vimentin, smoothelin and Smooth Muscle Actin (SMA) to differentiate myofibroblasts, MM and MP in 70 TUR-B specimens of BUC cases.[13] They found that vimentin expression in myofibroblasts was more numerous and stronger than in MM and MP. SMA was stained on MM and MP but could not distinguish MM and MP. Smoothelin's expression was significantly more and stronger in MP. From this study, it seems that it can be assumed that the use of SMA is less beneficial than vimentin and smoothelin.[13]

Before the researchers focused on using smoothelin and vimentin as markers, several studies examined many candidate markers. One of them is a previous study that reported a pilot study involving 5 RC specimens of BUC cases and 6 autopsy bladder specimens without abnormalities in the bladder.[5] There were 12 proteins studied as candidate markers, which were actin, caldesmon, type IV collagen, cytokeratin, desmin, elastin, fibronectin, filamin, laminin, myotilin, smoothelin, and vimentin. Of the 12 candidates, five have the potential to differentiate MM and MP (desmin, smoothelin, filamin, collagen type IV and vimentin). Desmin, smoothelin, filamin and type IV collagen were more strongly expressed in MP, while vimentin was more strongly expressed in MM. From the pilot study, it is recommended to use panel immunohistochemistry to differentiate MM and MP because, in some cases, the intensity of marker staining on MM and MP can be equivalent.[5]

The studies described above show that Smoothelin, desmin, and vimentin are the three best markers. Smoothelin and desmin were moderately expressed in MP, whereas vimentin was only expressed in MM and not expressed in MP. Ideally, all three have to be used as a panel. Still, in this study, smoothelin was chosen as the single marker, considering that smoothelin was highly expressed in intensity and quantity.

When compared with the results of the HE slide evaluation, a significant association was found between the results of HE and Smoothelin, with a p-value <0.001. Only 12 of 72 samples were discordant, of which 9 samples positive for smoothelin were diagnosed negative for HE, and 3 samples negative for smoothelin were diagnosed positive for HE. The HE results used here were the consensus of the five observers, where HE was categorized as positive if at least three observers interpreted it as positive. As a comparison, studies using a single marker have never been reported as in this study. However, almost all studies include smoothelin and consistently show that smoothelin was a good marker for identifying MP.[5,6,11-16]

However, this study has limitations. It has been unable to answer and solve problems related to negative results due to unsampled MP. In future studies, it is necessary to identify markers that can accurately predict the presence of MP invasion even though MP tissue is not visible on the slides.

CONCLUSION

There were two main conclusions of this study. First, the interobserver agreement level in assessing MP invasion on HE slides of TUR-B specimens for BUC cases at Prof. Dr. I.G.N.G. Ngoerah was low to moderate. Second, the consensus from the results of the MP invasion examination on HE slides by five observers had a significant positive association with the results of smoothellin immunohistochemistry.

As a suggestion, the significant positive association between the results of consensus HE examination and smoothellin indicates that HE would be better examined by several observers than individually to overcome subjectivity. If the laboratory has sufficient resources, the provision of smoothelin markers can be considered for better accuracy in assessing MP invasion in order to improve the management of BUC patients.

CONFLICT OF INTEREST

There is no competing interest regarding the manuscript.

ETHICS CONSIDERATION

Ethics approvals were obtained before the study was conducted from the Ethics Committee of the Faculty of Medicine Universitas Udayana (Letter no. 1061/UN14.2.2.VII.14/LT/2022) and receiving a research permit letter from the director of Prof. Dr. I.G.N.G. Ngoerah hospital (Letter no.LB.02.01/XIV.2.2.2/25608/2022).

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AUTHOR CONTRIBUTION

NWW contributes to concepts, design, the definition of intellectual, literature search, experimental study, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review and guarantor. NPS contributes to the design, the definition of intellectual, literature search, experimental study, data acquisition, data analysis, statistical analysis, and manuscript preparation. LPII contributes to experimental study, data acquisition, and data analysis. IMG contributes to experimental study, data acquisition, and data analysis. NMM contributes to experimental study, data acquisition, and data analysis. Tanaka contributes to literature search, data analysis, and statistical analysis. SK contributes to literature search, data analysis, and statistical analysis. IWY contributes to the design, definition of intellectual, literature search, clinical study, data analysis, and statistical analysis, manuscript preparation.

REFERENCES

1. Moch H, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, et al. The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol. 2022;82(5):458-468.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
3. Winarti NW, Widayanti LA. Clinicopathological Profile of Patients with Bladder Lesions in Sanglah Hospital during the Period 2013-2017. Gaceta Medica de Caracas. 2022;130(Suppl 1):S73–S78.
4. DeGeorge KC, Holt HR, Hodges SC. Bladder Cancer: Diagnosis and Treatment. Am Fam Physician. 2017;96(8):507-514.
5. Poletajew S, Wilczek E, Wasiutyński A, Górnicka B. Antigenic profile of muscularis mucosae and muscularis propria of the urinary bladder. Iranian Journal of Immunology. 2015;12(1):50–63.
6. Elkady N, Abdou AG, Kandil M, Ghanem N. Diagnostic value of smoothelin and vimentin in differentiating muscularis propria from muscularis mucosa of bladder carcinoma. International Journal of Biological Markers. 2017;32(3):e305–e312.
7. Heabah NAG, Aref MF, Mokhtar MA, Shareef MM, El-Enain, AMA. Is Smoothelin Useful for Detection of Muscularis Propria Invasion in Urinary Bladder Carcinoma Cases? International Journal of Current Microbiology and Applied Sciences. 2019;8(7):350-358.
8. Kamoun A, de Reynie`s A, Allory Y, Sjo ̈dahl G, Robertson AG, Seiler R, et al. A Consensus Molecular Classification of Muscle-invasive Bladder Cancer. European Urology. 2020;77(4):420–433
9. Poletajew S, Fus L, Walędziak M, Pomada P, CiechańSka J, Wasiutyński A, et al. Comparison of Pathological Staging and Grading of Urothelial Bladder Carcinoma in Post-transurethral Resection and post-radical Cystectomy Specimens. Pol J Pathol. 2014;65(4):305-312.
10. Mazzucchelli R, Marzioni D, Tossetta G, Pepi L, Montironi R. Bladder Cancer Sample Handling and Reporting: Pathologist's Point of View. Frontiers in Surgery. 2021;8:754741.
11. Mahde MM, Al Asadi AZ. Proper staging of bladder urothelial carcinoma using smoothelin and vimentinimmunohistochemical stains. Pakistan Journal of Medical and Health Sciences. 2021;15(1):412–416.
12. Mihai IM, Olteanu GE, Herman D, Anderco D, Iacob M, Nataras B, Cumpanas AA, et al. Analysis of Tumor Depth Invasion With Anti-Smoothelin Antibody in Equivocal Transurethral Resection of Urinary Bladder Tumor Surgical Specimens. International Journal of Surgical Pathology. 2021;29(1):54–63.
13. Kinra P, Malik A, Sivaramakrishna B, Dutta SV. Role of Immunohistochemistry in Differentiating Muscularis Mucosae from Muscularis Propria as An Aid to Accurate Staging of Invasive Urothelial Carcinoma : A Pilot Study. Advance Laboratory Medicine International. 2015;5(1):4–15.
14. Muhar AM, Siregar DR, Prabisma D. Relationship of gallbladder histopathology towards types of stones in cholelithiasis patients at Universitas Sumatera Utara Hospital, Medan, Indonesia. Bali Medical Journal. 2021;10(1):1-3.
15. Tafwid MI, Pratama ARH. Association between patient with muscle-invasive bladder cancer with the incidence of urinary tract infection in Karawang Regional Hospital: a single center study. Bali Medical Journal. 2022;11(3):1494-1497.
16. Santosa KB, Tirtayasa PMW, Oka AAG. Urological complications following obstetric-gynecologic procedures at Sanglah General Hospital, Bali-Indonesia. Bali Medical Journal. 2018;7(2):480-484.