Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://dx.doi.org/10.21608/zumj.2022.133357.2555 Volume 28, Issue 6, November 2022(1414-1429)

Manuscript ID ZUMJ-2204-2555 (R1)
DOI **10.21608/zumj.2022.133357.2555**

ORGINAL ARTICLE

Prognostic relevance of immunohistochemical expression of Sox2, YAP1, and IMP3 in non-muscle invasive bladder cancer

Authors:

1- *Hanaa M. Ibrahim: MD of Pathology, Faculty of Medicine, Zagazig University ZAGAZIG, EGYPT.*
2- *Aziza E. Abdelrahman: MD of Pathology, Faculty of Medicine, Zagazig University ZAGAZIG, EGYPT.*
3- *Amira Elwan: MD of Clinical Oncology and nuclear medicine, Faculty of Medicine, Zagazig University, Egypt.*
4- *Eman Elsebai: MD of Clinical Oncology and nuclear medicine, Faculty of Medicine, Zagazig University, Egypt.*
5- *Ahmed M. Eliwa: Urolology, Faculty of Medicine, Zagazig University, Egypt.*
6- *Samah S. Elbasateeny: MD of Pathology, Faculty of Medicine, Zagazig University, ZAGAZIG, Egypt*

The corresponding author:
* Aziza E. Abdelrahman: MD Pathology, Faculty of Medicine, Zagazig University
Address: Pathology Department, Zagazig , Egypt.

E-mail address of the corresponding author: azaelsayed@gmail.com

Telephone number of corresponding author: 002-01068743218

Submit Date 2022-04-18
Revise Date 2022-05-02
Accept Date 2022-05-09

ABSTRACT

BACKGROUND: Despite advances in intravesical therapy and surgery, up to 30% of NMIBC suffer progression to MIBC. **OBJECTIVES:** To identify the valuable prognostic biomarkers, we analyzed the immunohistochemical expression of Sox2, YAP1, and IMP3 in 60 cases of NMIBC who underwent TURBT with adjuvant intravesical bacillus-Calmette-Guerin (BCG). **METHODS**: The immunohistochemical expression was done on a sixty patients who complained from primary papillary superficial transitional cell carcinoma (TCC) of the urinary bladder (Stage Ta-T1).Their predicting role for recurrence, progression, progression-free survival (PFS), and recurrence-free survival (RFS) was assessed. **RESULTS**: High Sox2 expression was observed in 55% of NMIBC cases, and it was significantly associated with the tumor size, grade, and stage ($p < 0.001$ for each). High YAP1 was noted in 33.3% of the cases, and its expression was significantly associated with the tumor size, grade, and stage ($p < 0.001$ for each). Strong IMP3 expression was detected in 45% of the cases, and it was associated with the tumor size, grade, and stage ($p < 0.001$ for each). Analysis of follow-up period revealed that NMIBC with high Sox2, high YAP1, and Strong IMP3 expression exhibited a potent relation with tumor recurrence, progression, and shorter RFS & PFS.

CONCLUSIONS: High Sox2, high YAP1, and strong IMP3 could be considered as adverse prognostic factors of tumor recurrence and progression in NMIBC, and these patients should be followed carefully. Therefore, we suggest that Sox2, YAP1 and IMP3 should be considered and evaluated during the selection of the appropriate management strategy for NMIBC patients.

Keywords: Sox2; YAP1; IMP3; Immunohistochemistry; Non-muscle-invasive bladder cancer; Prognostic markers

INTRODUCTION

Bladder cancer (BC) is the 3rd most common neoplasm in males and the 11th most common neoplasm in females. In Egypt, it comprises 30% of all cancer cases with an incidence rate of 13.5/100,000 individuals according to the National Cancer Institute (1). Nearly 70% are diagnosed as non-muscle-invasive bladder cancer (NMIBC) with a recurrence rate of 60% within the first year of initial diagnosis (2). Up to 30% of NMIBC will progress to invasive disease, causing substantial burden on patients and healthcare systems (3). The

unpredictability of prognosis in NMIBC with identical features may reflect its variations in molecular backgrounds. Therefore, detection of molecular biomarkers for monitoring recurrence and progression is needed (4). This has become a very interesting area of research.

The endoscopic resection and adjuvant intravesical therapy are the preferred protocol for NMIBC, depending on the risk classification. High-risk patients who failed to respond to the adjuvant therapy constitute a challenging clinical situation to manage (5). Therefore, an accurate prediction of progression is critically important in the management of NMIBC. Tumor grade and stage have been shown to be significant predictors for progression however their predictive abilities of are still insufficient (6). Thus, more features are needed to improve the prognostic accuracy.

Bladder Cancer could be regarded as a stem cell disease (7). Several cancer stem cell (CSC) markers have been identified in BC and were associated with tumor initiation, maintenance of stemness, progression, recurrence and metastasis (8). Among these stem cell markers are Sox2 and YAP1 biomarkers (7).

Yes-associated protein-1 (YAP1) and Sex-determining region Y (SRY)-box 2 (Sox2) have been studied for their possible association with CSC traits. However, their oncogenic mechanism in bladder cancer remains unclear (9).

Sox2 is a transcription factor that belongs to the SRY-related HMG-box (SOX) family. It plays an essential role in regulating developmental processes (10). Aberrant expression of Sox2 has been correlated with the presence of CSCs in many types of cancers. Several studies concluded that the role of Sox2 in different solid tumors was not only to act as a trigger of carcinogenesis but also promotion of invasion, migration, and metastasis (10). Expression of Sox2 refers to a subpopulation of tumor cells that fuel the growth of established BC while its expression is absent in normal urothelial cells (11).

YAP1 is a transcriptional coactivator which doesn't contain a DNA-binding domain. It interacts with transcription factors such as TEA domain (TEAD1–4) proteins binding to genes' promoters. It is a core component of the Hippo signaling pathway in mammals which is a key regulator of cell growth, tissue homeostasis, and organ size (12).

Activation of Hippo pathway phosphorylates and sequesters YAP1 in the cytoplasm by LATS1/2 kinases and prevenst its translocation to the nucleus. The phosphorylated YAP1 remains in the cytoplasm; and its function as a transriptional co-activator is thereby inhibited as translocation of YAP1 to nucleus is a necessary step for its function as transcriptional coactivator (13). It was reported that YAP1 mutation can inhibit its phosphorylation leading to accumulation of the oncogenic YAP1 in the nucleus (14). YAP1 trafficking to the nucleus has been reported to contribute to BC progression and relapse (15). Mechanistically, YAP1 contributes to urothelial tumor growth via Sox2 (9).

Sox2 and YAP1 promotes AKT phosphorylation in bladder cancer cells by activation of Insulin growth factor (IGF) signaling (12,16). This signaling is important in the maintenance of stemmness of cancer cells through regulating self-renewal, pluripotency, and EMT (17). These data suggest that YAP1 and Sox2 play distinct roles in bladder cancer cells survival.

Insulin like growth factor II mRNA binding protein (IMP3) is a member of the insulin-like growth factor II messenger RNA binding protein (IMP) family(4). IMP family members are important for cell proliferation, migration, stability and RNA transportation, through a high specific binding to target mRNAs during early processes of embryogenesis (18). IMP3 has been reported to contribute to tumorigenesis in several human cancers (19,20). However, the biological functions of IMP3 in bladder cancer are poorly understood (19).

Hence, there is an actual need for reliable tumor markers to predict BCG response and NMIBC progression to invasive tumor, and so customize patients' treatment. This is the first study that investigates the combined expression of YAP1, Sox2, and IMP3 in NMIBC. Their predicting value in NMIBC regarding; the tumor progression, recurrence, and the disease-free survival was analyzed.

METHODS

Patients

A sixty patients who complained from primary papillary superficial transitional cell carcinoma (TCC) of the urinary bladder (Stage Ta-T1 ; with or without CIS) were studied in this study. Surgical resection of trans-urethral resection of bladder tumor (TURBT) was done in the Urology department from January 2017 to December 2019. According to American urological association (AUA), the intermediate and high-risk patients were treated by TURBT, with standard regimen of BCG.

The formalin-fixed-paraffin-embedded tissue specimens were collected from Pathology department of the same institute. The hematoxylin &

eosin-stained slides were reviewed and the cases were graded according to 2018 WHO tumor classification and AJCC Staging System, 8th edition was used to identify the stages (21,22). The clinical data as the age, sex, date of diagnosis, multifocality, treatment strategy, cystoscopy follow-up was reported in Urology and Clinical Oncology departments. Cases that had a recurrent tumor, isolated Tis, incomplete clinical information, uncertain follow-up data or absent detrusor muscle in biopsy were excluded.

Follow-up of the patients was done by cystoscopy and urinary cytology every 3 months for 2 years and then every 6 months. Recurrence was defined as occurrence of a new tumor of the same T stage and grade confirmed by biopsy after first complete TURBT. Progression was defined as recurrence a new tumor of higher T stage and grade, muscle invasion or distant metastasis confirmed by biopsy. Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University (ZU-IRB: 9169).

Therapy regimen

According to the current guidelines (23) , intravesical BCG instillation was given for only 50 cases (intermediate or high-risk cases) after two-four weeks post-endoscopy. For 6 weeks; the dose of BCG instillation therapy was 80 mg (Tokyo 172 strain) that continued weekly. After that, BCG instillation therapy was administrated every three months for at least one year. Twenty-one patients underwent chemo-radiation at time of progression. Cisplatin was proposed in cisplatin eligible cases without extensive CIS.

Immunohistochemistry

The immunohistochemical staining procedure was performed according to the polymer Envision detection system. The Primary antibodies that used were : anti-Sox2 (1:400; rabbit polyclonal antibody, MA, USA), IMP3 rabbit polyclonal antibody (0.1mg/ml concentration, Chongqing Biopsies Company, Cat No YPA1463, China) at a dilution of 1:100 and YAP1 rabbit monoclonal (1:2000; ab205270, Abcam, Cambridge, UK. Diaminobenzidine substrate was used as the chromogen. Mayer's hematoxylin was used to counter stain the slides .In each cycle of doing IHC, positive controls [squamous cell carcinoma larynx for SOX2, prostate carcinomas for YAP1and pancreatic carcinoma for IMP3] were included and negative controls were performed by omitting the primary antibodies.

Assessment of immunohistochemistry Sox2 scoring

Sox2 immunhistochemical expression is detected in the nucleus of tumor cells mainly. We score the positive tumor cells as : 0: negative; 1: <25%; 2: 25–50%; 3: 51–75%; and 4: >75%. Then, the staining intensity score was analyzed as: (0: negative; 1: weak; 2: moderate; and 3: strong). Both scores were added to identify a final score, which ranged from 0 to 7, with a cutoff point of 4. Negative/low expression was less than 4 scores and high expression: 4–7 scores (24).

YAP1 scoring

Nuclear staining intensity was graded as: 0 for negative, 1 for weak, 2 for moderate, or 3 for strong staining. The percentage was graded as: 0 for 0-5% positive cells, 1 for 6-25% positive cells, 2 for 26-50% positive cells, 3 for 51-75% positive cells, and 4 for 76-100% positive cells. Finally, the score was calculated by multiplying the staining intensity score by the fraction of positive cells score. A final score ≥ 6 was estimated as high expression (25).

IMP3 scoring

Dark brown cytoplasmic staining of the tumor cells were defined as positive. The intensity was scored as 0: negative; 1: weak; 2: moderate; and 3: strong. The percentage score was assigned as follows: 0% to 5%, 0 points; 6% to 25%, 1 point; 26% to 50%, 2 points; 51% to 75%, 3 points; and 76% to 100%, 4 points. A final score was calculated by multiplying both scores and staining scores were categorized as weak (<6) and strong (≥6) (4).

Statistics

Categorical variables were expressed as a number (percentage) and the continuous variables were expressed as the mean ± SD & median (range), and Continuous data checked for normality by using Shapiro Walk test. The two groups of non-normally distributed data were compared using Mann-Whitney U test . Pearson's Chi-square test or Fisher's exact test were used to compare the Percent of categorical variables when was appropriate. NMIBC Recurrence Free Survival (NMIBC-RFS) was calculated as the time elapse between date of TURBT and date of NMIBC recurrence or the most recent follow-up contact that patient was known as NMIBC recurrence free. Time to progression to MIBC (TTP to MIBC) was calculated as the time elapse between date of TURBT and date of MIBC recurrence or the most recent follow-up contact that patient was known as MIBC recurrence free. Overall Survival (OS) was calculated as the time elapse between date of diagnosis and date of death or the most recent

follow-up contact (censored). Stratification of NMIBC-RFS, TTP to MIBC and OS was done according to immunohistochemistry markers were estimated using the method of Kaplan-Meier plot was use to estimate, those time-to-event distributions and compared using two-sided exact log-rank test. All tests were two sided. A p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (IBM Corp., Armonk, NY, USA) and MedCalc Statistical Software version 18.9.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

RESULTS

Clinicopathological criteria of the studied NMIBC cases (Table 1):

The clinicopathological criteria of sixty NMIBC patients were listed in Table 1. Fourty-five men (75%) and 15 women (25%) were included . The mean age of these patients was 51.1 year (range 35-64 year). The predominant tumor size was > 3 cm (60%). Concomitant CIS and multifocality were noted in 26.7%, 28.3% of the cases respectively. Low grade tumors were identified in 36.7% while high grade were in 63.3% of the cases (Figure 1). Before BCG; pathologic stage included pTa in 35% and pT1 in 65% of the examined cases. The mean follow-up duration was 45.7 months (range 24-48 months) and the tumor recurrence and progression were detected in 15 patients (25%) and 17 patients (28.3%), respectively. At time of progression and recurrence, 21 patients underwent concurrent chemo-radiation and 11 patients underwent radical cystectomy.

Sox2, YAP1, and IMP3 expression in the examined NMIBC cases (Table 2, 3):

Sox2 immunoexpression was high in 55% of our NMIBC cases (Fig. 1), there was a significant up-regulation of Sox2 immunohistochemical expression with tumor size, histological grade, and tumor stage ($p< 0.001$ for each) . However, there was no significant relation between Sox2 immunohistochemical expression and both CIS and multiplicity of NMIBC could be detected.

YAP1 immunohistochemical expression was high in 20 cases of examined NMIBC cases (33.3%) (Fig. 2) . A significant relation was estimated between YAP1 up-regulation with tumor size, histological grade, and tumor stage ($p< 0.001$ for each). On the other hand, no significant relation between the presence of CIS or the multiplicity of NMIBC and YAP1 expression could be detected.

Strong IMP3 immunohistochemical expression was detected in 27 cases of NMIBC (45%) (Fig. 2). Up-regulation of IMP3 immunohistochemical expression was significant with tumor size, histological grade, and tumor stage ($p< 0.001$ for each). Unlike, there was a non-significant association of IMP3 immunohistochemical expression with CIS or the multiplicity of NMIBC. There was a strong relation between Sox2 expression and both YAP1 and IMP3 expresssion (<0.001 for each).

Prognostic value of Sox2, YAP1, and IMP3 expression in the examined NMIBC cases (Table 4):

Recurrence of NMIBC was noted in 15 cases during the follow-up period. A significant relation between the high expression of Sox2, YAP1, and IMP3 and recurrence of the tumor was approved ($p = 0.001$, $p <0.001$, $p <0.001$ respectively).

On the other hand, 17 cases (28.3%) progress to MIBC withihout response to BCG treatment. Furthermore, there was a significant association of Sox2, YAP1, and IMP3 expression and tumor progression ($p= 0.001$, $p <0.001$, $p= 0.002$ respectively). RFS and PFS were stratified according to Sox2, YAP1, and IMP3 expression and demonstrated in Kaplan-Meier plot curves . Kaplan-Meier curve with log-rank test showed that high Sox2, high YAP1, and strong IMP3 were associated with shorter OS ($p= 0.002$, 0.006 and 0.003 respectively), shorter RFS and PFS (Fig 3).

Table (1): Clinicopathological features, immunohistochemical markers and outcome of 60 patients with NMIBC.

Characteristics	All patients (N=60)		Characteristics	All patients (N=60)	
	No.	%		No.	%
Age (years)			Sox2		
Mean±SD	51.11	±6.63	Low	27	45%
Median (Range)	51.50	(35 – 64)	High	33	55%
Sex			YAP1		
Male	45	75%	Low	40	66.7%
Female	15	25%	High	20	33.3%
Size			IMP3		
≤3cm	24	40%	Weak	33	55%
>3cm	36	60%	Strong	27	45%
T stage			Follow-up duration (months)		
Ta	21	35%	Mean±SD	45.70	±5.23
T1	39	65%	Median (Range)	48	(24 – 48)
Grade			Treatment at progression and recurrence	(N=32)	
Low grade	22	36.7%	Cystectomy	11	34.4%
High grade	38	63.3%	Chemoradiation	21	65.6%
CIS			NMIBC Recurrence	(N=60)	
Absent	44	73.3%	Absent	45	75%
Present	16	26.7%	Present	15	25%
Multiplicity			Progression to MIBC		
Absent	43	71.7%	Absent	43	71.7%
Present	17	28.3%	Present	17	28.3%
AUA			Mortality	(N=60)	
Low risk	10	16.7%	Alive	46	76.7%
Intermediate risk	11	18.3%	Died	14	23.3%
High risk	39	65%			

Table (2): Relation between clinicopathological features and immunohistochemical staining for Sox2, YAP1, IMP3 in NMIBC patients (N=60).

Characteristics	All patients (N=60)		Sox2				p-value	YAP1				p-value	IMP3				p-value
			Low (N=27)		High (N=33)			Low (N=40)		High (N=20)			Weak (N=33)		Strong (N=27)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Size																	
≤3cm	24	(40%)	21	(87.5%)	3	(12.5%)	<0.001[a]	23	(95.8%)	1	(4.2%)	<0.001[a]	21	(87.5%)	3	(12.5%)	<0.001[a]
>3cm	36	(60%)	6	(16.7%)	30	(83.3%)		17	(47.2%)	19	(52.8%)		12	(33.3%)	24	(66.7%)	
T stage																	
Ta	21	(35%)	20	(95.2%)	1	(4.8%)	<0.001[a]	21	(100%)	0	(0%)	<0.001[a]	20	(95.2%)	1	(4.8%)	<0.001[a]
T1	39	(65%)	7	(17.9%)	32	(82.1%)		19	(48.7%)	20	(51.3%)		13	(33.3%)	26	(66.7%)	
Grade																	
Low grade	22	(36.7%)	21	(95.5%)	1	(4.5%)	<0.001[a]	22	(100%)	0	(0%)	<0.001[a]	20	(90.9%)	2	(9.1%)	<0.001[a]
High grade	38	(63.3%)	6	(15.8%)	32	(84.2%)		18	(47.4%)	20	(52.6%)		13	(34.2%)	25	(65.8%)	
CIS																	
Absent	44	(73.3%)	22	(50%)	22	(50%)	0.197[a]	28	(63.6%)	16	(36.4%)	0.409[a]	24	(54.5%)	20	(45.5%)	0.907[a]
Present	16	(26.7%)	5	(31.2%)	11	(68.8%)		12	(75%)	4	(25%)		9	(56.2%)	7	(43.8%)	
Multiplicity																	
Absent	43	(71.7%)	20	(46.5%)	23	(53.5%)	0.708[a]	26	(60.5%)	17	(39.5%)	0.105[a]	23	(53.5%)	20	(46.5%)	0.708[a]
Present	17	(28.3%)	7	(41.2%)	10	(58.8%)		14	(82.4%)	3	(17.6%)		10	(58.8%)	7	(41.2%)	
AUA																	
Low risk	10	(16.7%)	10	(100%)	0	(0%)	<0.001[a]	10	(100%)	0	(0%)	<0.001[a]	10	(100%)	0	(0%)	<0.001[a]
Intermediate risk	11	(18.3%)	10	(90.9%)	1	(9.1%)		11	(100%)	0	(0%)		9	(81.8%)	2	(18.2%)	
High risk	39	(65%)	7	(17.9%)	32	(82.1%)		19	(48.7%)	20	(51.3%)		14	(35.9%)	25	(64.1%)	
Sox2																	
Low	27	(45%)						26	(96.3%)	1	(3.7%)	<0.001[a]	23	(85.2%)	4	(14.8%)	<0.001[a]
High	33	(55%)						14	(42.4%)	19	(57.6%)		10	(30.3%)	23	(69.7%)	
YAP1																	
Low	40	(66.7%)	26	(65%)	14	(35%)	<0.001[a]						31	(77.5%)	9	(22.5%)	<0.001[a]
High	20	(33.3%)	1	(5%)	19	(95%)							2	(10%)	18	(90%)	
IMP3																	
Weak	33	(55%)	23	(69.7%)	10	(30.3%)	<0.001[a]	31	(93.9%)	2	(6.1%)	<0.001[a]					
Strong	27	(45%)	4	(14.8%)	23	(85.2%)		9	(33.3%)	18	(66.7%)						

Table (3): immunohistochemical coexpression of Sox2, YAP1, and IMP3 in the studied caes.

IHC of the three markers	All studied NMIBC patients (N=60)	
	No.	%
Low Sox2/Low YAP1/Weak IMP3	23	38.3%
Low Sox2/Low YAP1/Strong IMP3	3	5%
Low Sox2/High YAP1/Weak IMP3	0	0%
High Sox2/Low YAP1/Weak IMP3	8	13.3%
Low Sox2/High YAP1/Strong IMP3	1	1.7%
High Sox2/Low YAP1/Strong IMP3	6	10%
High Sox2/High YAP1/Weak IMP3	2	3.3%
High Sox2/High YAP1/Strong IMP3	17	28.3%

Table (4): Relation between immunohistochemical staining for Sox2, YAP1, IMP3 and outcome in NMIBC patients (N=60).

Outcome	All patients	Sox2		p-value	YAP1		p-value	IMP3		p-value
	No. (%)	Low No. (%)	High No. (%)		Low No. (%)	High No. (%)		Weak No. (%)	Strong No. (%)	
NMIBC Recurrence	(N=60)	(N=27)	(N=33)		(N=40)	(N=20)		(N=33)	(N=27)	
Absent	45 (75%)	26 (96.3%)	19 (57.6%)	0.001[a]	39 (97.5%)	6 (30%)	<0.001[a]	33 (100%)	12 (44.4%)	<0.001[a]
Present	15 (25%)	1 (3.7%)	14 (42.4%)		1 (2.5%)	14 (70%)		0 (0%)	15 (55.6%)	
Recurrence Free Survival										
Mean (months) (95%CI)	43.26months (40.94–45.58)	46.81months (44.53–49.09)	40.07months (36.57–43.57)	<0.001[c]	47.36months (46.14–48.59)	34.59months (29.95–39.22)	<0.001[c]	48months	36.75months (32.46–41.04)	<0.001[c]
2-year NMIBC-RFS	87.7%	96.3%	80.2%		97.4%	67.3%		100%	70.6%	
3-year NMIBC-RFS	83.8%	96.3%	72.9%		97.4%	55.1%		100%	61.2%	
4-year NMIBC-RFS	71.5%	96.3%	48.8%		97.4%	16.3%		100%	32.9%	
Progression to MIBC										
Absent	43 (71.7%)	25 (92.6%)	18 (54.5%)	0.001[a]	35 (87.5%)	8 (40%)	<0.001[a]	29 (87.9%)	14 (51.9%)	0.002[a]
Present	17 (28.3%)	2 (7.4%)	15 (45.5%)		5 (12.5%)	12 (60%)		4 (12.1%)	13 (48.1%)	
Progression-Free Survival										
Mean (months) (95%CI)	43.19 months (40.93–45.45)	45.11 months (42.57–47.65)	41.27 months (37.84–44.70)	0.028[c]	43.76 months (41.38–46.14)	42.06 months (36.99–47.14)	0.027[c]	44.06 months (41.77–46.34)	42.73 months (38.55–46.91)	0.028[c]
2-year PFS	89.6%	96.2%	84.2%		92.5%	83.5%		93.9%	84.3%	
3-year PFS	83.9%	88.5%	80.4%		84.8%	83.5%		87.9%	79%	
4-year PFS	66%	80.8%	46.8%		66.8%	56.8%		63.6%	46.8%	
Overall Survival										
Mean (months) (95%CI)	45.70 months (44.38–47.01)	47.48 months (46.48–48.47)	44.24 months (42.12–46.35)	0.002[c]	46.60 months (45.31–47.88)	43.90 months (41.08–46.72)	0.006[c]	47.39 months (46.65–48.13)	43.63 months (41.06–46.19)	0.003[c]
2-year OS	98.3%	100%	97%		100%	95%		100%	96.3%	
3-year OS	90%	96.3%	84.8%		92.5%	85%		100%	77.8%	
4-year OS	76.7%	96.3%	60.6%		87.5%	55%		90.9%	59.3%	

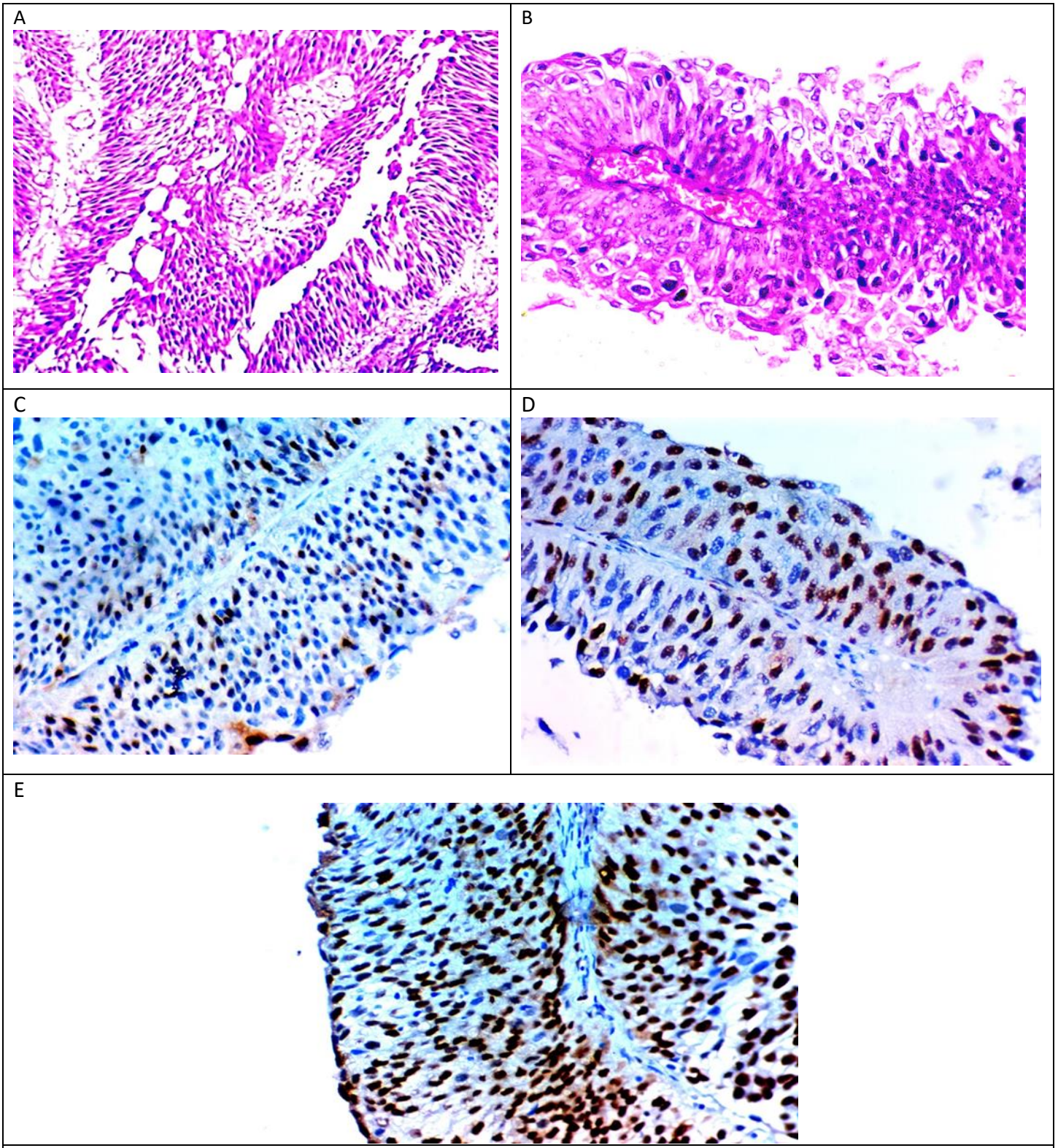

Fig. 1. (A) A case of low-grade non-muscle invasive bladder cancer (H& E ×100), **(B)** A case of high-grade non-muscle invasive bladder cancer (H& E ×400). **(C)** A case of low-grade NMIBC showing low nuclear Sox2 immunoreactivity (IHC ×400), **(D)** A case of high-grade NMIBC showing high nuclear Sox2 (IHC ×400), **(E)** A case of low-grade NMIBC showing high nuclear Sox2 (IHC ×400).

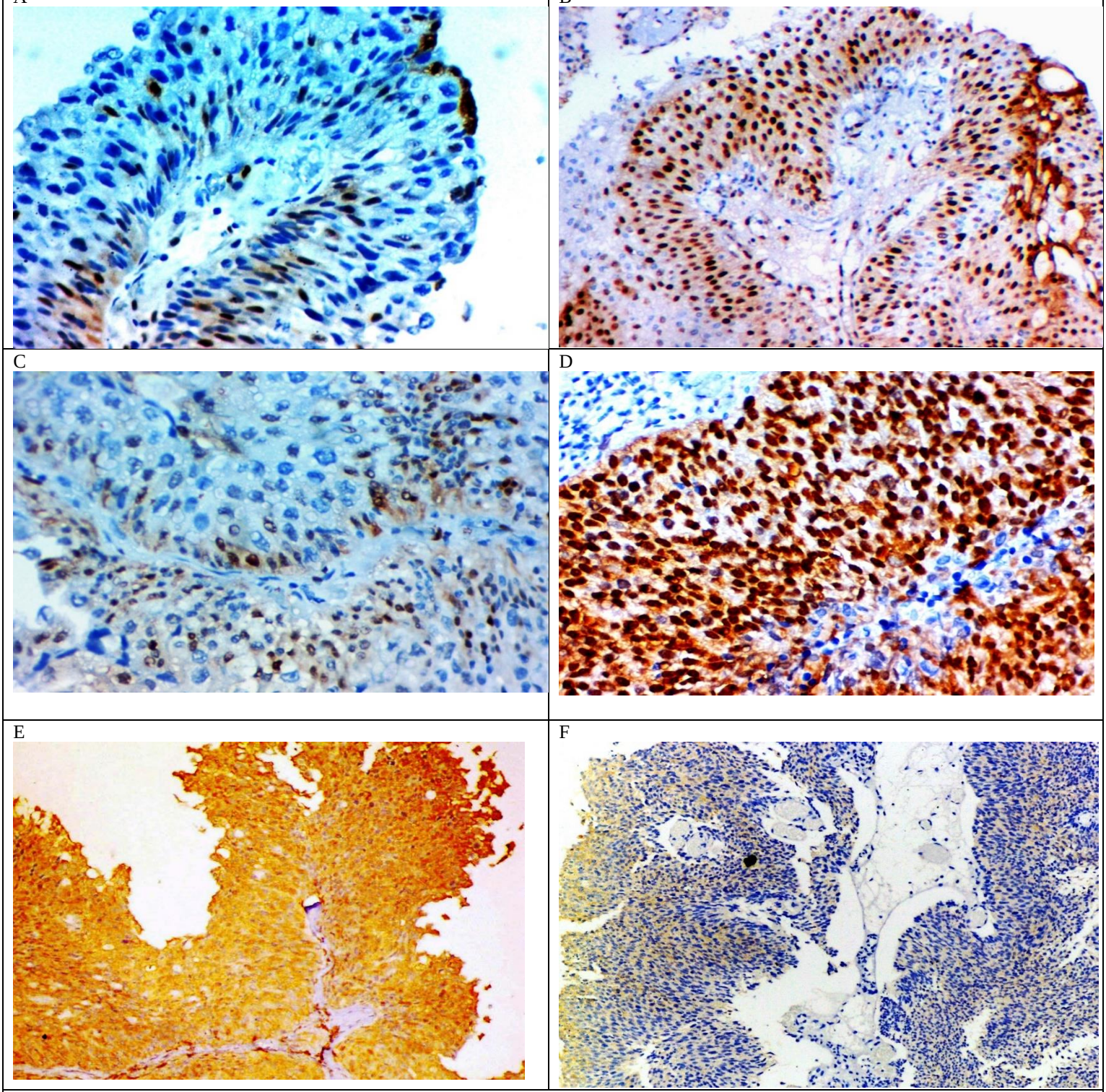

Fig. 2. (A) A case of high-grade NMIBC showing low nuclear with lesser cytoplasmic YAP1 expression (IHC ×400), **(B)** A case of high-grade NMIBC showing high nuclear YAP1 expression (IHC ×400), **(C)** A case of low-grade NMIBC showing low nuclear YAP1 (IHC ×100), **(D)** A case of low-grade NMIBC showing high nuclear YAP1 expression (IHC ×400). **(E)** A case of high-grade NMIBC showing strong cytoplasmic IMP3 expression (IHC ×400), **(F)** a case of low-grade NMIBC showing weak cytoplasmic IMP3 expression (IHC ×200)

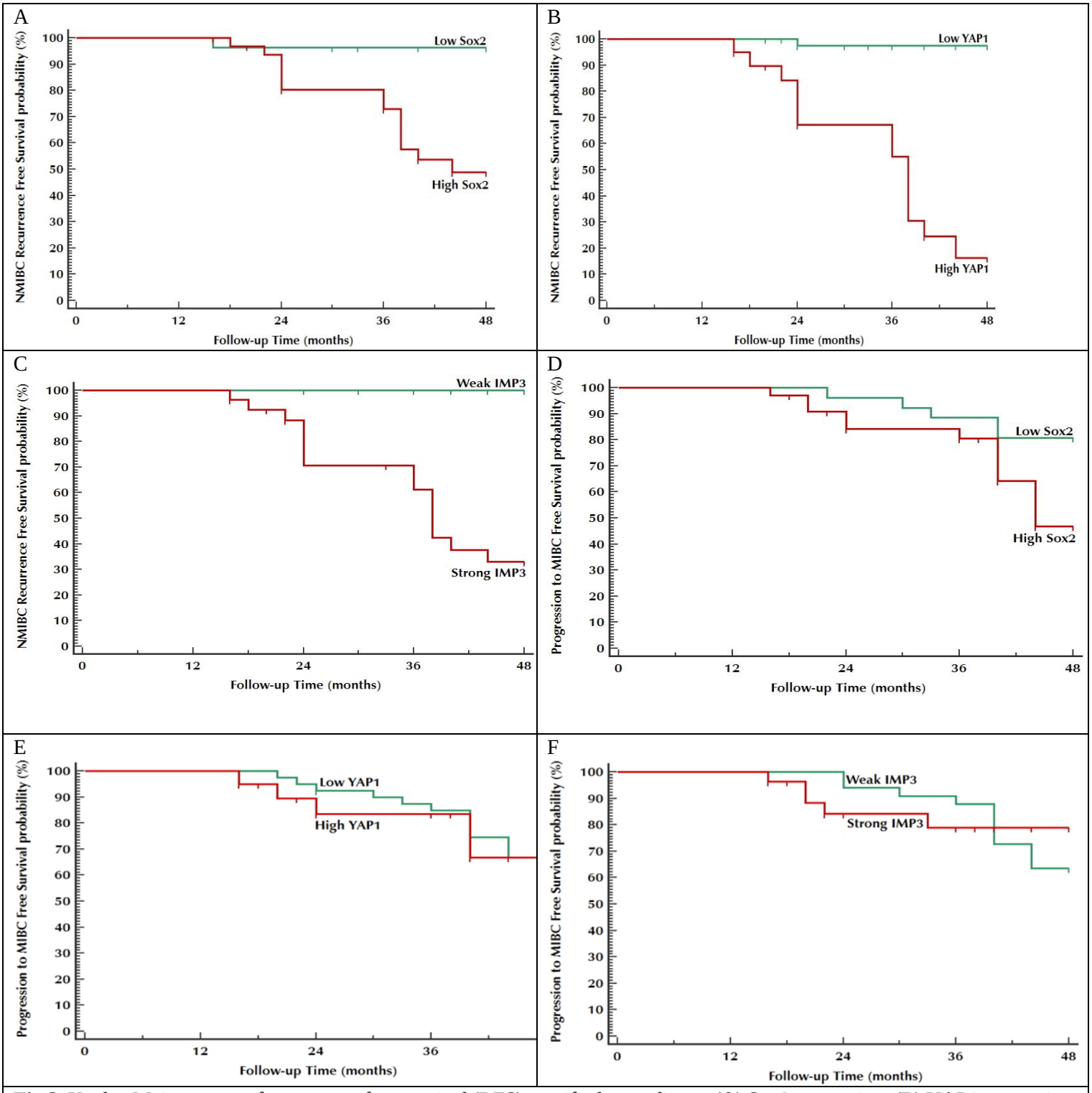

Fig.3. Kaplan Meier curves of recurrence-free survival (RFS) stratified according to **(A)** Sox2 expression, **(B)** YAP1 expression, and **(C)** IMP3 expression. Kaplan Meier curves of progression-free survival (PFS) stratified according to **(D)** Sox2 expression, **(E)** YAP1 expression, and **(F)** IMP3 expression.

DISCUSSION

Despite advances in intravesical therapy and surgery, up to 30% of NMIBC will progress to MIBC. There is a challenge to select patients who can benefit from an early radical surgical intervention. However, there are few clinicopathological prognostic biomarkers that can classify the high-risk subgroups that need early radical cystectomy (3).

BC stem cells revealed a heterogeneous molecular signature , that markedly influences their response to therapy. So understanding the BC stemness is necessary to improve their treatment (7). In the present study we investigate the immunohistochemical expression of Sox2, YAP1, and IMP3 to identify their predictive role of recurrence and progression in NMIBC.

Sox2 was characterized as a marker for stem-like tumor cells in bladder cancer (11). Our cases revealed a high nuclear Sox2 expression in 55% of cases in line with Ruan et al (26), who observed that 53.97 % of 126 NMIBC had a high Sox2 expression. In contrast to our results, Fekry and colleagues reported that Sox2 expression was negative or low in 100% of their cases (27). Furthermore, Bayoumi et al noted that only 6% of the studied cases had a high Sox2 expression of NMIBC (28). These different results may be related to the different specificity and sensitivity of the commercially available antibodies used in these studies and different scoring methods In Bayoumi et al study, a cutoff level of 5% was used to separate the tumors into a negative and positive Sox2 expression group (28).

The localization of Sox2 expression was nuclear in our study which was approved previously (28,29). We noted that Sox2 expression was significantly associated with tumor size, tumor grade and stage in NMIBC confirming the previous observation of the vital role of Sox2 expression in tumor progression. Previously, Sox2+ cells were isolated from BC tissue with the ability to regrowth secondary tumors in vivo and spheres in vitro. Sox2 promotes pluripotency and therefore may initiate carcinogenesis (26,28). It was analyzed that there was a significant association between Sox2 expression and higher histological grade of esophageal carcinoma (30). Accordingly, the results on the role of Sox2 expression in cancer bladder were matched with that in other cancers. However, some results were contradictory, for example, high Sox2 in MIBC was not correlated with tumor grade and stage (28,29). Their observations could be aimed to their case selection, as their cases were MIBC (29).

Sox2 is an important regulator of self-renewal in embryonic stem cell. Regarding the cancer stem cell (CSC) theory, the overexpression of Sox2 is potentially involved in carcinogenesis and could affect tumor recurrence and metastasis (26). Unlike Bayoumi et al, we identified that high Sox2 expression was associated with tumor recurrence and progression to MIBC (28). Also, there was a significant association with shorter RFS as well as shorter PFS as previously reported (26), where high Sox2 expression had significantly poorer recurrence-free survival when compared with patients with low Sox2 expression. Formerly, in the human ovarian carcinoma; it was approved that Sox2 overexpression correlates with the tumor recurrence (31). Sox2 is a key regulator of pluripotency in stem cells and has been linked to poor survival in various malignancies including BC (26,32).

Consequently, we supposed that Sox2 upregulation is a strong predictor of NMIBC progression to MIBC and so, it can be used as a target for novel therapeutic drugs. A potential correlation between Sox2 and YAP1 has been hypothesized . For example: YAP1 signaling contributes to maintaining Sox2 independent CSCs (33) and other tumorigenic pathways that are associated with aggressive tumor behavior. The inhibitioin of Sox2 suppressed YAP1-induced cancer stemness properties and tumor growth (9).

In this study, a strong asociation between Sox2 and YAP1 expression was estimated and their crucial role in tumor progression and recurrence was evaluated in the examined NMIBC patients. These findings suggest an essential role of these two combined molecules in the tumor invasiveness. This data was in line to Ooki et al who noted a positive correlation between the genetic expression of Sox2 and YAP1 in BC specimens where the overexpressed YAP1 cells exhibited a noticeable overexpression of Sox2 consistent with the increased sphere-forming and self-renewal abilities confirming the Yap1- Sox2 axis role in BC (9).

Previously, the role of YAP1 in regulating cellular stemness has been noted in many reports. In NSCLC, YAP1 directly interacts with OCT4 followed by Sox2 upregulation to facilitate self-renewal while depletion of YAP1 lowered the expression of core embryonic stem cell factors such as Sox2 (34). YAP1 associated biological features that related to CSCs are implemented through regulating Sox2 activity in BC (9).

YAP1, the nuclear effector of the Hippo signaling pathway has an important role in cell

growth, and its dysregulation is involved in pathogenesis of many human tumor including lung, colon, breast ,pancreatic, liver, ovarian, and prostate cancer (35). many

A few studies investigated YAP1 expression in NMIBC, and they have noted controversial observations. Latz and his colleagues reported that YAP1 was higher in normal urothelial bladder tissue than NMIBC without significant association with the prognosis, including tumor progression and recurrence (35). Latz et al evaluated both nuclear and cytoplasmic YAP1 as a prognostic factor in contrast to our study, where only the nuclear YAP1 was assessed. Previously it was reported that is rarely to see cytoplasmic expression of YAP without nuclear expression (36).

On the other hand, a parallel study had been reported by Liu et al that evaluated YAP1 in BC cases. They reported a higher YAP1 expression in BC than in normal urothelium which was in agree with our data. They noted that positive expression of YAP1 was correlated closely with tumor grade, and higher pT. Consequently, they suggested that YAP1 expression could be used as an additional tool in identifying patients at risk of progression, and it may also be useful in optimizing individual BC therapy management (37).

Previously, YAP1 was observed in (27.8%) and (45.8%) of NMIBC cases respectively (25,37). In the present study, YAP1 expression was high in 33.3% of the NMIBC cases with a significant association with tumor size, grade and stage . This confirms the essential role of YAP1 in EMT process in BC where the inhibition of YAP1 impaired the progression of EMT in BC. YAP1 gene, as an oncogene, has been shown to be a potent regulator of cell growth (25,37).

Positive staining of YAP1 in our work was nuclear in most cases and a few cases showed nuclear with a lesser cytoplasmic staining. Cytoplasmic expression was not considered in our evaluation of the tumor progression. Wu et al approved that nuclear YAP1 plays a vital role in human cancer (38) which was confirmed in BC by Ghasemi et al where it contributed to BC progression and relapse (15). In the present study a significant relation was noted between YAP1 expression with recurrence, progression, and poor RFS& PFS confirming the contribution of YAP1 overexpression to poor prognosis and progressive features of human urothelial carcinoma of the bladder (37).

This gives insight into the possible mechanisms which can be involved in the enhancement and alteration of YAP1 activity. The malignant cells express excess YAP1 during genomic amplification that might affect the normal physiologic regulatory systems leading to abnormal cytoplasmic accumulation. Cytoplasmic accumulation of YAP1 maintains a constant pool of the protein for nuclear translocation.Furthermore, the stability of the YAP1 protein is altered in the cancerous tissues resulting in ineffective protein turnover and excessive YAP1 activity (36).

It was reported that YAP1 enable the tumors cells to enter mitosis with unrepaired DNA through driving IGF signaling that contributes to maintenance of stem-like phenotype in cancer cells (12,17) and Sox2 regulates IGF signaling in bladder cancer cells (16). Here in the current study, we examined IMP3 which represents one family member of these signals which modulates their biological activities (39).

Previous studies indicated that IMP3 linked to advanced disease stage and adverse clinical outcome in several cancers (40,41). Xu et al study revealed up regulation of IMP3 in colorectal cancer that was associated with worse clinical outcome (40). Furthermore, IMP3 was found to be an oncogenic factor initiating the glioblastoma proliferation (41). However, the role of IMP3 in driving progression of bladder cancer has yet been elucidated.

In a previous investigation, 183 cases of NMIBC were evaluated and showed that IMP3 expression was strongly related to higher tumor grade and stage (4). In our study, IMP3 immunohistochemical expression was strong in 45% of the cases with a significant relation with tumor size, grade and stage that confirms the essential value of IMP3 in tumor cell proliferation, invasion, and the tumor progression as reported previously (4).

Furthermore, we identified strong IMP3 expression as a poor prognostic factor of the tumor progression relative to those with weak IMP3 expression. Strong IMP3 expression in the studied NMIBC was significantly related to the progression to MIBC and tumor recurrence with poor RFS as well as PFS as previously noted (4), where they showed that the progression of NMIBC to MIBC was significantly associated with the IMP3-strong group than with negative-IMP3 group with a significant association with PFS and DFS. On the other hand, Sitnikova et al reported that 60% of the patients with IMP3-positive superficial urothelial carcinomas developed metastases, compared with none of patients with IMP3-negative tumors.

Moreover, IMP3 was approved as a potential therapeutic biomarker for cancers (43) . Consequently, we suggested that IMP3 is an unfavorable prognostic marker that could classify a group of patients with a high potential to develop progression and who might benefit from early aggressive therapy and need intensive follow-up compared with patients of weak IMP3 expression.

Several clinical and pathological parameters such as, tumor stage, tumor grade, focality, concomitant carcinoma in situ (Cis), tumor size and number of recurrences have been suggested to be the most important risk factors in NMICB (6) . Thus, adding Sox2, YAP1, and IMP3 expression especially in patients with these risk factors could help in the decision between a bladder-sparing approach or radical cystectomy.

Conclusion

Our results suggest that high Sox2, high YAP1, and strong IMP3 expression were strongly associated with bad prognosis, poor survival, and these NMICB patients can undergo direct surgical radical cystectomy, while patients low Sox2, low YAP1, and weak IMP3 immunohistochemical expression could be treated with transurethral resection of the mass followed by postoperative intravesical BCG. Furthermore, we think that this study may give an assistance to physicians in recognizing cases with poor prognosis who may possibly benefit from early, radical surgery. More studies are needed to understand the pathways and molecular mechanisms through which Sox2, YAP1, and IMP3 affects the biology of NMIBC.

CONFLICT OF INTEREST

The authors report no conflicts of interest that are directly relevant to the content of this study.

List of abbreviations

SOX2: SRY-Box Transcription Factor 2.
YAP1 : yes-associated protein 1.
NMIBC: Non muscle invasive bladder cancer.
MIBC : Muscle invasive bladder cancer.
CIS : Carcinoma in situ .
TURBT : Transurethral resection of bladder tumor.
BCG: Bacillus-Calmette-Guerin.
TCC: transitional cell carcinoma.
PFS : Progression-free survival.
RFS : Recurrence-free survival.
BC : Bladder cancer.
CSC : cancer stem cell.
IGF : Insulin growth factor.
EMT : Epithelial-mesenchymal transition.
AKT : serine/threonine-specific protein kinases
NSCLC: Non-small-cell lung carcinoma

REFERENCES

1. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in egypt: results of the national population-based cancer registry program. J Cancer Epidemiol. 2014; 2014:437971. doi: 10.1155/2014/437971. Epub 2014 Sep 21. PMID: 25328522; PMCID: PMC4189936.
2. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM, et al. EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2017 Mar;71[3]:447-461. doi: 10.1016/j.eururo.2016.05.041. Epub 2016 Jun 17. PMID: 27324428.
3. Boegemann M, Krabbe LM. Prognostic Implications of Immunohistochemical Biomarkers in Non-muscle-invasive Blad Cancer and Muscle-invasive Bladder Cancer. Mini Rev Med Chem. 2020;20[12]:1133-1152. doi: 10.2174/1389557516666160512151202. PMID: 27173513.
4. Yang F, Zhou Q, Meng L, Xing N. IMP3 is a biomarker for non-muscle-invasive urothelial carcinoma of the bladder associated with an aggressive phenotype. Medicine [Baltimore]. 2019;98[27]:e16009. doi:10.1097/MD.0000000000016009.
5. Lenis AT, Lec PM, Chamie K, Mshs MD. Bladder Cancer: A Review. JAMA. 2020 Nov 17;324[19]:1980-1991. doi: 10.1001/jama.2020.17598. PMID: 33201207.
6. Fujii Y. Prediction models for progression of non-muscle-invasive bladder cancer: A review. Int J Urol. 2018 Mar;25[3]:212-218. doi: 10.1111/iju.13509. Epub 2017 Dec 16. PMID: 29247553.
7. Abugomaa A, Elbadawy M, Yamawaki H, Usui T, Sasaki K. Emerging Roles of Cancer Stem Cells in Bladder Cancer Progression, Tumorigenesis, and Resistance to Chemotherapy: A Potential Therapeutic Target for Bladder Cancer. Cells. 2020;9[1]:235. Published 2020 Jan 17. doi:10.3390/cells9010235
8. Li Y, Lin K, Yang Z, Han N, Quan X, Guo X, et al. Bladder cancer stem cells: clonal origin and therapeutic perspectives. Oncotarget. 2017 Jul 8;8[39]:66668-66679. doi: 10.18632/oncotarget.19112. PMID: 29029546; PMCID: PMC5630446
9. Ooki A, Del Carmen Rodriguez Pena M, Marchionni L, Dinalankara W, Begum A, Hahn NM, et al. YAP1 and COX2 Coordinately Regulate Urothelial Cancer Stem-like Cells. Cancer Res. 2018;78[1]:168-181. doi:10.1158/0008-5472.CAN-17-0836
10. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. Cell Stem Cell. 2013;12[1]:15-30. doi:10.1016/j.stem.2012.12.007
11. Zhu F, Qian W, Zhang H, Liang Y, Wu M, Zhang Y, et al. SOX2 Is a Marker for Stem-like Tumor Cells in Bladder Cancer. Stem Cell Reports. 2017 Aug

8;9[2]:429-437. doi: 10.1016/j.stemcr.2017.07.004. PMID: 28793245; PMCID: PMC5550032.
12. Szulzewsky F, Holland EC, Vasioukhin V. YAP1 and its fusion proteins in cancer initiation, progression and therapeutic resistance. Dev Biol. 2021 Jul;475:205-221. doi: 10.1016/j.ydbio.2020.12.018. Epub 2021 Jan 8. PMID: 33428889; PMCID: PMC8107117.
13. Godlewski J, Kiezun J, Krazinski BE, Kozielec Z, Wierzbicki PM, Kmiec Z. The Immunoexpression of YAP1 and LATS1 Proteins in Clear Cell Renal Cell Carcinoma: Impact on Patients' Survival. Biomed Res Int. 2018 Apr 3;2018:2653623. doi: 10.1155/2018/2653623. PMID: 29850494; PMCID: PMC5903336.
14. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev. 2007 Nov 1;21[21]:2747-61. doi: 10.1101/gad.1602907. PMID: 17974916; PMCID: PMC2045129.
15. Ghasemi H, Mousavibahar SH, Hashemnia M, Karimi J, Khodadadi I, Mirzaei F, et al. Tissue stiffness contributes to YAP activation in bladder cancer patients undergoing transurethral resection. Ann N Y Acad Sci. 2020 Aug;1473[1]:48-61. doi: 10.1111/nyas.14358. Epub 2020 May 19. PMID: 32428277.
16. Chiu YF, Wu CC, Kuo MH, Miao CC, Zheng MY, Chen PY, et al. Critical role of SOX2-IGF2 signaling in aggressiveness of bladder cancer. Sci Rep. 2020 May 19;10[1]:8261. doi: 10.1038/s41598-020-65006-z. PMID: 32427884; PMCID: PMC7237425.
17. Malaguarnera R, Belfiore A. The emerging role of insulin and insulin-like growth factor signaling in cancer stem cells. Front Endocrinol [Lausanne]. 2014 Feb 4;5:10. doi: 10.3389/fendo.2014.00010. PMID: 24550888; PMCID: PMC3912738.
18. Degrauwe N, Suvà ML, Janiszewska M, Riggi N, Stamenkovic I. IMPs: an RNA-binding protein family that provides a link between stem cell maintenance in normal development and cancer. Genes Dev. 2016;30[22]:2459-2474. doi:10.1101/gad.287540.116
19. Huang W, Li Y, Zhang C, Zha H, Zhou X, Fu B, et al. IGF2BP3 facilitates cell proliferation and tumorigenesis via modulation of JAK/STAT signalling pathway in human bladder cancer. J Cell Mol Med. 2020 Dec;24[23]:13949-13960. doi: 10.1111/jcmm.16003. Epub 2020 Oct 22. PMID: 33094561; PMCID: PMC7753985.
20. Gong Y, Woda BA, Jiang Z. Oncofetal protein IMP3, a new cancer biomarker. Adv Anat Pathol. 2014 May;21[3]:191-200.
21. Humphrey PA,Moch H,Cubilla AL,Ulbright TM,Reuter VE.The 2016 WHO classification of tumour so the urinary system and male genital organs—partB: prostate and bladder tumours.Eur Urol2016;70[1]:106–19.
22. Paner GP, Stadler WM, Hansel DE, Montironi R, Lin DW, Amin MB. Updates in the eighth edition of the tumor-node-metastasis staging classification for urologic cancers.Eur Urol2018;73[4]:560–9.
23. Babjuk M, Burger M, Zigeuner R, Shariat SF, van Rhijn BW, Compérat E, et al; European Association of Urology. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2013. Eur Urol. 2013 Oct;64[4]:639-53. doi: 10.1016/j.eururo.2013.06.003. Epub 2013 Jun 12. PMID: 23827737.
24. Gayyed MF, Tawfiek ER. Utility of SOX2 and Livin Co-Expression in the Prognosis of Bladder Cancer With Bilharzial and Non-Bilharzial Bladder Status. World J Oncol. 2015 Oct;6[5]:446-455. doi: 10.14740/wjon942w. Epub 2015 Oct 26. PMID: 28983346; PMCID: PMC5624670.
25. Chen X, Li S, Yu Z, Tan W. Yes-associated protein 1 promotes bladder cancer invasion by regulating epithelial-mesenchymal transition. Int J Clin Exp Pathol. 2019 Mar 1;12[3]:1070-1077. PMID: 31933921; PMCID: PMC6945140.
26. Ruan J, Wei B, Xu Z, Yang S, Zhou Y, Yu M, et al. Predictive value of Sox2 expression in transurethral resection specimens in patients with T1 bladder cancer. Med Oncol. 2013 Mar;30[1]:445. doi: 10.1007/s12032-012-0445-z. Epub 2013 Jan 10. PMID: 23307254.
27. Fekry MF, ElRehim DM, Elghany MI, Moussa RA. Clinicopathologic significance of SOX2 protein expression in bladder carcinoma. Egypt J Pathol [serial online] 2019 [cited 2021 Aug 14];39:364-72. doi: 10.4103/EGJP.EGJP_45_19.
28. Bayoumi D, Abdallah D, Hosney M. The role of immunohistochemical expression of OCT-4 and SOX2 as predictors of recurrence and progression in superficial urinary bladder carcinoma. Egypt J Pathol 2017;37:17–
27. doi:10.1097/01.XEJ.0000515976.40228.b9
29. Nottingham CU, Patel SG, Clark PE, DeGraff DJ, Gregg JR, Anderson BB, et al. SOX2 Expression in Patients who Underwent Radical Cystectomy for Urothelial Carcinoma of the Bladder. Clin Oncol. 2016; 1: 1022.
30. Wang Q, He W, Lu C, Wang Z, Wang J, Giercksky KE, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. Anticancer Res. 2009 Apr;29[4]:1233-41. PMID: 19414369.
31. Zhang J, Chang DY, Mercado-Uribe I, Liu J. Sex-determining region Y-box 2 expression predicts poor prognosis in human ovarian carcinoma. Hum Pathol. 2012 Sep;43[9]:1405-12. doi: 10.1016/j.humpath.2011.10.016. Epub 2012 Mar 7. PMID: 22401770; PMCID: PMC3374028.
32. Lundberg IV, Löfgren Burström A, Edin S, Eklöf V, Öberg Å, Stenling R, et al. SOX2 expression is regulated by BRAF and contributes to poor patient

prognosis in colorectal cancer. PLoS One. 2014 Jul 10;9[7]:e101957. doi: 10.1371/journal.pone.0101957. PMID: 25010701; PMCID: PMC4092103.
33. Schewe M, Franken PF, Sacchetti A, Schmitt M, Joosten R, Böttcher R, et al. Secreted Phospholipases A2 Are Intestinal Stem Cell Niche Factors with Distinct Roles in Homeostasis, Inflammation, and Cancer. Cell Stem Cell. 2016 Jul 7;19[1]:38-51. doi: 10.1016/j.stem.2016.05.023. Epub 2016 Jun 9. PMID: 27292189.
34. Bora-Singhal N, Nguyen J, Schaal C, Perumal D, Singh S, Coppola D, et al. YAP1 Regulates OCT4 Activity and SOX2 Expression to Facilitate Self-Renewal and Vascular Mimicry of Stem-Like Cells. Stem Cells. 2015 Jun;33[6]:1705-18. doi: 10.1002/stem.1993. PMID: 25754111; PMCID: PMC4441573.
35. Latz S, Umbach T, Goltz D, Kristiansen G, Müller SC, Ellinger J. Cytoplasmatic and Nuclear YAP1 and pYAP1 Staining in Urothelial Bladder Cancer. Urol Int. 2016;96[1]:39-45. doi: 10.1159/000438868. Epub 2015 Aug 20. PMID: 26287745
36. Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, et al. Expression of Yes-associated protein in common solid tumors. Hum Pathol. 2008 Nov;39[11]:1582-9. doi: 10.1016/j.humpath.2008.04.012. Epub 2008 Aug 13. PMID: 18703216; PMCID: PMC2720436.
37. Liu JY, Li YH, Lin HX, Liao YJ, Mai SJ, Liu ZW, et al. Overexpression of YAP 1 contributes to progressive features and poor prognosis of human urothelial carcinoma of the bladder. BMC Cancer. 2013 Jul 19; 13:349. doi: 10.1186/1471-2407-13-349. PMID: 23870412; PMCID: PMC3750259.
38. Wu Y, Hou Y, Xu P, Deng Y, Liu K, Wang M, et al. The prognostic value of YAP1 on clinical outcomes in human cancers. Aging [Albany NY]. 2019 Oct 15;11[19]:8681-8700. doi: 10.18632/aging.102358. Epub 2019 Oct 15. PMID: 31613226; PMCID: PMC6814621.
39. Chen P, Wang SJ, Wang HB, Ren P, Wang XQ, Liu WG, et al. The distribution of IGF2 and IMP3 in osteosarcoma and its relationship with angiogenesis. J Mol Histol. 2012 Feb;43[1]:63-70. doi: 10.1007/s10735-011-9370-2. Epub 2011 Nov 1. PMID: 22042095.
40. Xu W, Sheng Y, Guo Y, Huang Z, Huang Y, Wen D, et al. Increased IGF2BP3 expression promotes the aggressive phenotypes of colorectal cancer cells in vitro and vivo. J Cell Physiol. 2019 Aug;234[10]:18466-18479. doi: 10.1002/jcp.28483. Epub 2019 Mar 20. PMID: 30895618.
41. Suvasini R, Shruti B, Thota B, Shinde SV, Friedmann-Morvinski D, Nawaz Z, et al. Insulin growth factor-2 binding protein 3 [IGF2BP3] is a glioblastoma-specific marker that activates phosphatidylinositol 3-kinase/mitogen-activated protein kinase [PI3K/MAPK] pathways by modulating IGF-2. J Biol Chem. 2011 Jul 22;286[29]:25882-90. doi: 10.1074/jbc.M110.178012. Epub 2011 May 25. PMID: 21613208; PMCID: PMC3138258.
42. Vikesaa J, Hansen TV, Jønson L, Borup R, Wewer UM, Christiansen J, et al. RNA-binding IMPs promote cell adhesion and invadopodia formation. EMBO J. 2006 Apr 5;25[7]:1456-68. doi: 10.1038/sj.emboj.7601039. Epub 2006 Mar 16. PMID: 16541107; PMCID: PMC1440323.
43. Sitnikova L, Mendese G, Liu Q, Woda BA, Lu D, Dresser K, et al. IMP3 predicts aggressive superficial urothelial carcinoma of the bladder. Clin Cancer Res. 2008 Mar 15;14[6]:1701-6. doi: 10.1158/1078-0432.CCR-07-2039. PMID: 18347170.

To Cite:
Ibrahim, H., Abdelrahman, A., Elwan, A., Elsebai, E., Eliwa, A., Elbasateeny, S. Prognostic relevance of immunohistochemical expression of Sox2, YAP1, and IMP3 in non-muscle invasive bladder cancer. *Zagazig University Medical Journal*, 2022; (1414-1429): -. doi: 10.21608/zumj.2022.133357.2555