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The Bulletin publishes basic and clinical research original articles, reviews, editorials, case reports, and letters to the editor relevant to urooncology (prostate cancer, urothelial cancers, testis and kidney cancer, benign prostatic hyperplasia, and any aspect of urologic oncology). The Bulletin of Urooncology is indexed by several international databases and is committed to rigorous peer review.

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3) Main text,

4) Acknowledgements (optional),

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A. Original Research Articles

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Introduction

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- Results
- Discussion
- Study Limitations
- Conclusion
- Acknowledgements
- References
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Preparation of research articles, systematic reviews, and meta-analyses must comply with study design guidelines: CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consortstatement.org/);

PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/);

STROBE statement, a checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/);

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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Case reports should include cases which are rarely seen and distinctive in diagnosis and treatment. These can include brief descriptions of

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Introduction

Main Text

Conclusions

Tables/Figures

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Full title (in English and in Turkish); Turkish title will be provided by the editorial office for authors who are not Turkish speakers

Authors' names and institutions

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Any grants or financial support received for the paper

B. Abstract and Keywords

Abstracts should be prepared in accordance with the specific instructions for the different article types. For original articles, a structured abstract should be provided using the following headings: Objective, Materials and Methods, Results, and Conclusions. Provide 3-5 keywords. English keywords should be provided from Medical Subject Headings (http://www.nlm.nih.gov/mesh).

C. Main Text

Introduction: Should include brief explanation of the topic, the objective of the study, and supporting information from the literature.

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Book Chapters: Lang TF, Duryea J. Peripheral Bone Mineral Assessment of the Axial Skeleton: Technical Aspects. In: Orwoll ES, Bliziotes M, eds. Osteoporosis: Pathophysiology and Clinical Management. New Jersey, Humana Pres Inc, 2003;83-104.

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Contents

Original Articles

Reviews

Original Article

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Comparison of Lidocaine and Prilocaine Efficiencies in Periprostatic Nerve Block for Transrectal Prostate Biopsy: A Randomized Prospective Study

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Abstract

Objective: Periprostatic nerve block (PPNB) and intrarectal local anesthesia (IRLA) are two of the most commonly used methods for pain control in the transrectal ultrasonography (TRUS)-guided prostate needle biopsy procedure, which is the standard method for the diagnosis of prostate cancer. This study compared the efficacy of IRLA alone and with PPNB using lidocaine or prilocaine.

Materials and Methods: A total of 64 patients with suspicious rectal examination symptoms and/or serum prostate-specific antigen (PSA) elevation underwent standard 12-core TRUS-guided prostate needle biopsy. The patients were divided into 3 groups: IRLA with lidocaine gel only (group 1), IRLA with lidocaine gel plus PPSB with 2% lidocaine injection (group 2), and IRLA with lidocaine gel plus PPSB with 2% prilocaine injection (group 3). Patients' pain levels were assessed by visual analog scale (VAS) immediately (VAS-1) and at 45 minutes (VAS-2) after prostate biopsy. In addition, serum PSA levels, age, body mass index (BMI), prostate volume, and cancer detection rates of the patients were recorded.

Results: There were no statistical differences between the groups in terms of total PSA, age, BMI, prostate volume, or cancer detection rates. According to VAS-1 score, group 1 had more severe pain compared to the other groups, while there was no significant difference between groups 2 and 3. When VAS-2 score was examined, group 1 experienced the most severe pain, while group 3 was the least painful group. When asked which biopsy step was the most painful, all groups reported that introduction of ultrasound probe into the rectum was the most painful.

Conclusion: The combination of IRLA and PPNB seems to be a more effective method than IRLA alone for pain control during TRUS-guided prostate biopsy. Prilocaine and lidocaine have comparable onset but the effect of prilocaine lasts longer. Therefore, prilocaine is a preferable agent for prostate biopsy under TRUS guidance.

Keywords: Pain, anesthesia, biopsy, prostate

Introduction

Prostate cancer is the most common malignancy among elderly men and the second leading cause of cancer deaths in men after lung cancer (1,2). Transrectal ultrasonography (TRUS)-guided prostate needle biopsy is used as a standard method in the diagnosis of prostate cancer. Although this technique is minimally invasive, it can cause the patient severe pain both during and after the procedure (3). Pain usually occurs when the ultrasound probe is introduced into the rectum, during probe manipulations, and when the biopsy needle contacts the prostate (4).

Studies report that 65-90% of patients feel discomfort due to the procedure (5,6). Some studies have shown that this discomfort involves moderate to intolerably severe pain (7). Therefore, pain control during biopsy is an importance issue. Methods currently employed for pain control during prostate biopsy include intrarectal local anesthetic (IRLA), periprostatic nerve block (PPNB), caudal block, sedation anesthesia, and spinal anesthesia, with PPNB being the most widely preferred. However, there is no clear consensus on which method to use (8,9,10). Lidocaine and prilocaine are amide local anesthetics commonly used in urology practice. Although both are in the moderate-acting class, lidocaine has faster onset and shorter duration of action than prilocaine (11). In terms of patient comfort, it is important that the local anesthetic agent used in prostate biopsy has faster onset of action and provides long-term pain control. Although there are many previous studies

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in which lidocaine was used in PPNB, there are few studies comparing the effectiveness of prilocaine and lidocaine (12,13). This randomized prospective study was conducted to compare the effectiveness of two different active ingredients and three different methods used for pain control during and after TRUS-guided standard 12-core prostate biopsy.

Materials and Methods

Patient Selection: A total of 68 patients who underwent TRUS-guided prostate biopsy in our clinic between January 2015 and December 2015 with suspicion of prostate cancer were included in the study. Indications for prostate biopsy were specified as suspicious findings on digital rectal examination and/or elevated prostate-specific antigen (PSA) level for the patient's age. Exclusion criteria were previous history of prostate biopsy, presence of active urinary tract infection, acute prostatitis, bleeding diathesis, history of anorectal diseases such as anal fissure and/or hemorrhoid, and neurological disorders such as paraplegia or hemiplegia that can cause hypoesthesia or paresthesia. Patients with known history of allergy to the drugs used in the study were also excluded. For those using anticoagulants or antiaggregants that may cause bleeding disorder, the relevant department was consulted and their medications were discontinued at an appropriate interval before the procedure.

Ethical Considerations

Approval was obtained from the local ethics committee (Erciyes University Clinical Researches Committee, number: 2014/570) before the study. All patients were informed verbally and in writing and gave written informed consent before the procedure.

Patient Preparation and Study Design

For antibiotic prophylaxis, 750 mg ciprofloxacin was administered orally at 12-hour intervals. All patients started antibiotic therapy the day before the procedure and biopsy was performed after the third dose. No bowel preparation was performed before the procedure.

Patients included in the study were randomized into three groups according to their order of presentation. Biopsy was performed with only rectal gel containing 0.2 g lidocaine for patients in group 1, rectal gel containing lidocaine + periprostatic injection of 2% lidocaine in group 2, and rectal gel containing lidocaine + periprostatic injection of 2% prilocaine in group 3. A total of 2 grams of lidocaine-containing gel was applied to the rectal area 5 minutes before the procedure.

Biopsy procedures were performed by a single surgeon using transrectal probe with an EnVisor-C ultrasonography device (Philips, Eindhoven, Netherlands). In patients receiving periprostatic injections, the anesthetic was administered with an 18-gauge (G) 30 cm long needle into the area of the neurovascular bundle between the base of the prostate and seminal vesicles in the TRUS sagittal plane. At the beginning of the procedure, 2 mL ampules of 2% lidocaine or prilocaine were diluted with 8 mL of physiological saline solution and the resulting 10 mL solution was administered equally to the right and left periprostatic areas. Standard 12-core prostate biopsy was performed in all patients using 18-G 30 cm long biopsy needles. During the biopsy procedure, the patient was notified verbally when inserting and manipulating the probe and when first inserting the needle into the prostate. General pain experienced by the patients during the procedure was assessed immediately after the biopsy using a visual analogue scale (VAS) by a researcher who did not participate in the biopsy procedure, and this score was recorded as VAS-1. The patients were also asked at which stage of the procedure they felt the most severe pain, and this information was recorded. Pain was assessed again 45 minutes after the end of the biopsy procedure and recorded as VAS-2 score. In the VAS, patients scored their pain between 0 and 10, with a score of 0 indicating no pain at all and a score of 10 indicating very severe and intolerable pain.

Statistical Analysis

SPSS 22.0 (IBM Corp, Armonk, NY, USA) software was used for statistical analyses of the study data. Numerical data were assessed for normal distribution with Shapiro-Wilk test and histograms. Normally distributed numerical data were evaluated using one-way ANOVA with post-hoc Tukey test. Non-normally distributed numerical data were evaluated using Kruskal-Wallis test with post-hoc Bonferroni test. Categorical data were analyzed using chi-square test. A p value <0.05 was considered statistically significant.

Results

Of the 68 patients included in the study, 4 were excluded because they could not tolerate the procedure due to pain, despite appropriate anesthetic administration. None of the patients had drug-related early allergic reaction or life-threatening adverse effects. The mean age of the patients included in the study was 64.6±7.2 years and their median PSA level was 12 (1-165) ng/mL. There were no significant differences between the groups in terms of age, total PSA, body mass index, cancer detection rates, or prostate size (Table 1). Cancer was detected in 31 (48%) of the 64 patients, while pathology was benign in the other 33 patients (52%). A total of 768 biopsy cores were collected. Cancer was detected in 132 of those cores, for a per-core detection rate of 17%. Comparison of VAS-1 scores (obtained immediately after the biopsy procedure) between the groups showed that group 1 had significantly higher values compared to the other groups. In terms of VAS-2 scores (obtained 45 minutes after the end of the procedure), both group 1 and group 2 had significantly higher values than group 3. Relationships between the VAS scores of the groups are summarized in Table 2.

Patients in all groups reported that probe insertion was the most painful stage of the biopsy procedure (Figure 1).

Discussion

Despite being a minimally invasive diagnostic procedure, the pain experienced during TRUS-guided prostate biopsy leads to some difficulties and reservations for patients (4). Many anesthesia methods such as the administration of IRLA, PPNB, caudal block, sedation anesthesia, and spinal anesthesia are used

to increase patient comfort and compliance and to minimize pain (9,10). However, there is still no clear consensus on which method to use. In their study conducted with 96 patients, Rodriguez et al. (14) compared the effectiveness of PPNB and IRLA in patients who underwent TRUS-guided prostate biopsy. They used the active ingredient lidocaine in both groups and reported that patients who underwent PPNB had significantly less pain. According to the findings of Alavi et al. (15), PPNB with lidocaine was more advantageous compared with IRLA alone. In a meta-analysis comparing PPNB and IRLA with their combination, it was reported that the combination was more effective at controlling pain compared to IRLA or PPNB alone (16). There are numerous studies demonstrating that PPNB is effective in pain control during TRUS-guided prostate biopsy (17,18). However, some authors have argued that there IRLA and PPNB do not differ in terms of pain (12,19). In a 2004 study with 328 patients, Mallick et al. (19) compared patients who underwent biopsy with intrarectal lidocaine gel or PPNB with lidocaine. There was no difference between the groups in terms of the pain experienced during the biopsy procedure, but their patients reported that the application of PPNB was much more painful than the application of rectal gel. Based on these data, the researchers argued that IRLA, which is a less invasive method, is more advantageous.

All of the patients in our study received IRLA. In addition to IRLA, one group of patients also had PPNB with lidocaine and another had PPNB with prilocaine. This grouping allowed us to determine which method most effectively reduces pain during the procedure and compare which anesthetic agent can provide longer pain control after the procedure. Based on our results, PPNB in addition to IRLA resulted in more effective pain control than IRLA alone. Similarly, we compared the effectiveness of IRLA + lidocaine injection and IRLA + prilocaine injection in pain control using VAS scores. Immediately after the procedure (VAS-1), there was no statistically significant difference between the group that received periprostatic lidocaine injection and the group that received periprostatic prilocaine injection. However, at 45 minutes after the procedure (VAS-2), the group injected with prilocaine had significantly lower mean VAS score than the other two groups. The absence of a significant difference in VAS-1 scores between the lidocaine and prilocaine groups indicates that prilocaine and lidocaine have comparable onset of action. However, the significant difference in VAS-2 in group 3 suggests that prilocaine provides pain control for a longer time than lidocaine, and is more effective in patient comfort and pain palliation in the late post-prostate biopsy period. In 2005, Başar et al. (12) compared the effectiveness of PPNB with 1% prilocaine and 1% lidocaine injection. Contrary to our findings, they reported no significant differences in efficacy between the two groups based on comparison of VAS scores. In another study using a different active ingredient evaluated the efficacy of levobupivacaine in PPNB injection and showed that this agent provided better pain control compared to a diclofenac suppository alone (20).

The pain caused by transrectal prostate biopsy is attributed to introduction of the ultrasound probe into the rectum, probe manipulation, and contact between the biopsy needle and the prostate (7). In a recent meta-analysis analyzing 26

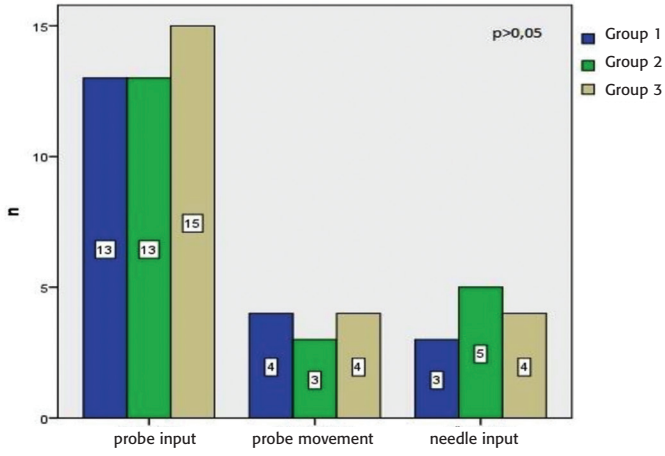

Figure 1. Distribution of groups according to most painful stage of procedure

Table 1. Demographic and clinical data of the patients by group

	Group 1 (n=20)	Group 2 (n=21)	Group 3 (n=23)	p
Age (years)	63.8±5.8	64.9±6.9	64.0±8.7	0.845*
Total PSA (ng/mL)	11.5 (8.25-21.75)	13 (8-21)	9 (7-30)	0.826+
BMI (kg/m^2)	26.3 (25-27)	25.5 (24.2-28)	23.7 (21.4-29)	0.689+
Prostate volume (mm^3)	45 (40.5-60)	50 (45-60)	45 (40-60)	0.591+
Cancer detection (n, %)	8/20 (40%)	12/21 (57%)	11/23 (48%)	0.546#

PSA: Prostate-specific antigen, BMI: Body mass index, *: One-way ANOVA, +: Kruskal-Wallis, #: Chi-square Data were presented as mean ± standard error and median (25th-75th quartiles)

Table 2. VAS-1 and VAS-2 values of the groups

	Group 1 (n=20)	Group 2 (n=21)	Group 3 (n=23)	p*
VAS-1	7.5 (7-8)^a	4 (2.5-5)^b	5 (3-6)^b	<0.001
VAS-2	4 (3-5)^a	3 (1.5-4)^a	2 (1-2)^b	<0.001

VAS-1: Visual analog scale for pain applied immediately after the biopsy procedure, VAS-2: Visual analog scale for pain applied 45 minutes after the biopsy procedure, *: Kruskal-Wallis, Post-hoc Bonferroni,
Values annotated with letters were significantly different from other values in the row, data were presented median (25th-75th quartiles)

studies, no significant difference was observed between the IRLA group and intrarectal placebo gel group in terms of pain during probe manipulations, whereas the IRLA group experienced less pain during needle penetration into the prostate. Similarly, comparison of lidocaine injection with PPNB and periprostatic placebo injection revealed that patients who had PPNB experienced less pain during needle penetration. Comparison of PPNB and PPNB + IRLA showed that probe manipulations were less painful in patients who received PPNB alone, while the PPNB + IRLA group experienced less pain when the needle contacted the prostate (16). When the patients in our study were asked which stage of the procedure was most painful, they said it was insertion of the rectal probe (Figure 1). However, because the different stages of the procedure were not evaluated with VAS, it is not possible to make a clear interpretation of the relationship between biopsy pain and anesthesia method.

In the present study, we observed no significant difference between the groups in terms of cancer detection rates. According to our results, anesthesia method and level of pain experienced during biopsy do not affect the biopsy result. Bolat et al. (21) also argued that there was no correlation between pain and pathology results based on their series of TRUS-guided transrectal prostate biopsies performed with PPNB. In another recent study, IRLA and PPNB results were compared in terms of pain and pathology results (22). That study also used VAS scoring and showed that the IRLA group had significantly higher pain levels than the PPNB group, whereas the cancer detection rate was higher in the PPNB group. The authors attributed this to the biopsy technician's inability to adequately manipulate the probe and effectively biopsy regions of the prostate where cancer is more likely to occur, such as the apical and far-lateral regions, when patients are in pain during the procedure.

Study Limitations

Limitations of our study include the small number of patients and lack of a control group given a placebo. In addition, pain was not evaluated separately with VAS for each step of the biopsy because it was not considered practical. Finally, we did not use anxiety scales to evaluate the patients' anxiety, which may affect pain threshold.

Conclusion

Our study showed that PPNB + IRLA is more effective than IRLA alone for pain control in TRUS-guided prostate biopsy, which is the gold standard in the diagnosis of prostate cancer. Prilocaine is similar to lidocaine in terms of speed of onset but provides longer-term pain control, suggesting that prilocaine is preferable for prostate biopsy pain management. However, randomized, prospective studies with larger sample size are required on this subject.

Ethics

Ethics Committee Approval: Erciyes University Clinical Researches Committee, number: 2014/570) before the study.

Informed Consent: All patients were informed verbally and in writing and gave written informed consent before the procedure.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.D., G.G., Design: A.D., G.G., Data Collection or Processing: A.D., G.G., Analysis or Interpretation: G.S., N.B., Literature Search: Ş.T.T., Writing: G.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Predicting Lamina Propria Invasion in Patients with Non-muscle-invasive Bladder Cancer: Do RDW and NLR Really Work?

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Abstract

Objective: To determine the role of preoperative neutrophil-to-lymphocyte ratio (NLR) and red cell distribution width (RDW) in predicting lamina propria invasion in bladder cancer.

Materials and Methods: Eighty-eight patients with non-muscle-invasive bladder cancer were evaluated retrospectively. The patients were divided into those with Ta tumors (group 1: n=36) and those with T1 tumors (group 2: n=52). For each patient, white blood cell, neutrophil, and leukocyte counts and RDW values were evaluated.

Results: NLR was significantly lower in patients with Ta tumors. In addition, NLR below 3.22 was associated with 80.6% probability of Ta disease. RDW sensitivity in Ta non-muscle-invasive bladder tumors was much higher compared to T1 tumors. RDW below 15.35 was associated with 94.4% probability of Ta disease.

Conclusion: NLR and RDW are basic blood parameters that physicians can assess easily. Our results indicate that a combination of NLR and RDW can help clinicians predict lamina propria invasion in non-muscle-invasive bladder tumors.

Keywords: Bladder cancer, superficial, lamina propria, invasion

Introduction

The incidence of bladder cancer has increased in recent years. Its prevalence increases with age and it is 3-4 times more common in men than in women. It is the sixth most common cancer in the United States of America and accounts for 8% of all cancers in men and 2% of all cancers in women (1). It is predicted that 76,960 new cases will be diagnosed and 16,390 deaths will be attributed to bladder cancer in 2016 (2). Seventy-five percent of newly diagnosed cases are limited to the mucosa (Ta, carcinoma *in situ*) or submucosa (T1), and these superficial bladder tumors have a high risk of recurrence and progression despite local treatment. The remaining 25% of newly diagnosed cases have muscle invasion and require a more radical approach such as surgery or radiotherapy (3).

Due to the high risk of recurrence and progression, risk scales have been developed to guide treatment planning. The European Organization for Research and Treatment of Cancer (EORTC) and Club Urológico Español de Tratamiento Oncológico (CUETO) risk models are most commonly used to predict a patient's risk of recurrence and progression. Although these tables are used frequently in clinical practice, they tend to overestimate the risks and have a low differentiation rate for prognostic results. As a result, new prognostic markers are needed to improve the predictive value of these risk evaluation tables (4). Previous studies have demonstrated the effect of systemic inflammation in the growth and progression of many types of cancer and shown that inflammation stimulates tumor angiogenesis, invasion, and metastasis (5). For example, in colorectal cancer patients who underwent potentially curative

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resection, systemic inflammatory response predicted poor outcome (6). A study by Kum et al. (7) showed that the inflammatory marker neutrophil-to-lymphocyte ratio (NLR) can be used to differentiate benign lesions from malign ones, especially for laryngeal lesions. Similar studies reported that NLR could be elevated in laryngeal cancer and may be a beneficial marker for diagnosis, detection of recurrence, and differentiation of malign and pre-malign lesions (8). Mano et al. (9) emphasized that elevated NLR was an independent predictor of progression and recurrence of non-muscle-invasive bladder cancers. In addition, NLR was shown to be associated with muscle invasion, extravesical disease, and worse disease-free and overall survival (10,11). Red cell distribution width (RDW) was also shown to be an independent prognostic factor in various cancers (12,13). Aim of this study was to determine the role of preoperative NLR and RDW in predicting lamina propria invasion in bladder cancer.

Materials and Methods

This study was approved by a local ethics committee and conducted in accordance with the principles of the Declaration of Helsinki. The medical files of 88 patients diagnosed with non-muscle-invasive bladder cancer between January 2014 and January 2017 were evaluated retrospectively. Patients with proven preoperative infection, unexplained leukocytosis, or history of hematological malignancy, and those who were re-operated due to bladder cancer were excluded from the study. The patients included in the study were separated into two groups, those with Ta tumors (group 1: n=36) and T1 tumors (group 2: n=52). For each patient, white blood cell (WBC), neutrophil (N), and lymphocyte (L) counts and RDW values were evaluated and postoperative pathology reports were reviewed. Groups were compared according to their age, gender, WBC, N and L counts, NLR, and RDW values.

Statistical Analysis

Statistical analyses were performed using SPSS software package (version 22.0, SPSS Inc. Chicago, IL, USA; licensed to Hitit University). Distribution of normality was tested with the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation or median (min-max) according to distribution, and categorical variables were expressed as numbers and percentages. Continuous variables were analyzed with Student's t-test to compare the means of normally distributed variables between two independent groups. Non normally distributed independent samples were compared using Mann-Whitney U test. Logistic regression analysis was performed with an enter method to identify independent predictors of the Ta and T1 groups. Relationships between categorical variables were investigated by chi-square test. Receiver operating characteristic (ROC) curve analyses were conducted and areas under the ROC curves were calculated to evaluate the diagnostic accuracy of RDW and NLR in detecting lamina propria invasion. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive likelihood ratio were calculated for optimal cutoff values. A p value <0.05 was used to determine the statistical significance.

Results

Patient characteristics (age, gender, tumor grade and size, origin, neutrophil count, lymphocyte count, platelet count, NLR, and RDW) are given Table 1.

After logistic regression analysis made using the Enter method; RDW and NLR were found to be statistically significant in differentiating T1 from Ta (p=0.023 and p=0.050 respectively; Table 2).

ROC analysis was performed to measure the classifying success of the logistic regression analysis. Area under curve was found to be 0.624 (0.507-0.742) and 0.600 (0.481-0.719) for RDW and NLR, respectively (Table 3). Cutoff points were determined as 15.35 for RDW and 3.22 for NLR by Youden index.

Table 1. Patient characteristics

	-	Group 1 (Ta)	Group 2 (T1)	p value
Age, years	-	70.89±10.67	69.67±12.33	0.632[b]
Gender	Female (%)	1 (11.1%)	9 (88.9%)	0.075[a]
	Male (%)	35 (44.9%)	43 (55.1%)	
Grade	High	6 (12.2%)	43 (87.8%)	**<0.001[a]**
	Low	30 (78.9%)	8 (21.1%)	
Tumor size	>3	11 (25.0%)	33 (75.0%)	**0.002[a]**
	<3	25 (58.1%)	18 (41.9%)	
Origin	Unifocal	25 (47.2%)	28 (52.8%)	0.171[a]
	Multi-focal	11 (32.4%)	23 (67.6%)	
Neutrophil count, median (minimum-maximum)	-	5.21 (2.66-12.60)	4.69 (1.80-15.21)	0.466[c]
Lymphocyte count, mean ± SD	-	1.98±0.73	1.95±0.71	0.842[b]
NLR, median (minimum-maximum)	-	2.40 (0.88-4.71)	2.84 (1.39-18.55)	0.114[c]
Platelet count, median (minimum-maximum)	-	241500 (97000-509000)	233000 (30000-489000)	0.561[c]
RDW, median (minimum-maximum)	-	13.5 (10.7-16.40)	14.0 (12.60-29.40)	0.049[c]

SD: Standard deviation, NLR: Neutrophil to lymphocyte ratio, RDW: Red cell distribution width, [a]chi-square test, [b]Student's t-test, [c]Mann-Whitney U test

Table 2. Logistic regression analysis results for estimation of Ta-T1 groups

	B	SE	p value	OR	95% CI for OR	
					Lower	Upper
RDW	0.361	0.159	**0.023***	**1.435**	1.050	1.960
Lymphocyte	1.034	0.695	0.137	2.812	0.721	10.971
Neutrophil	-0.510	0.273	0.062	0.600	0.351	1.025
NLR	0.983	0.502	**0.050***	**2.672**	1.000	7.143
Platelets	0.000	0.000	0.782	1.000	1.000	1.000

OR: Odds ratio, SE: Standard error of beta coefficient, CI: Confidence interval, RDW: Red cell distribution width, NLR: Neutrophil to lymphocyte ratio

Table 3. Receiver operating characteristic curve results

	RDW	NLR
AUC (95 %CI)	0.624 (0.507-0.742)	0.600 (0.481-0.719)
Cut-off	≤15.35	≤3.22
Sensitivity	0.294 (0.179-0.440)	0.451 (0.313-0.595)
Specificity	0.944 (0.800-0.990)	0.806 (0.634-0.912)
PPV	0.882 (0.622-0.979)	0.767 (0.572-0.894)
NPV	0.485 (0.365-0.607)	0.509 (0.374-0.642)
LR+	5.29 (1.28-21.73)	2.32 (1.12-4.82)

AUC: Area under curve, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likelihood ratio, CI: Confidence interval, RDW: Red cell distribution width, NLR: Neutrophil to lymphocyte ratio

Discussion

Non-muscle-invasive bladder cancer accounts for 70% of all bladder cancers. Recurrence and progression of superficial bladder tumors, which is a very heterogeneous group, is basically related to tumor grade, number of tumor foci, and tumor size. EORTC risk classification is a tool to predict prognosis of superficial bladder cancer, but recent studies have shown that new risk predictors are needed to improve this prognostic risk classification (14,15).

NLR and RDW are practical, easy to use, and accessible laboratory methods. In our study, we evaluated the role of NLR and RDW in predicting lamina propria invasion in patients with superficial bladder tumor who have undergone TUR.

Inflammation associated with cancer has been evaluated in many studies. Essentially, inflammation and cancer are linked by intrinsic and extrinsic mechanisms (5). Intrinsic mechanisms are related to tumor suppressor gene deactivation and oncogene activation, whereas the extrinsic mechanisms are activated via chronic inflammation or infection (16). Some prognostic scoring systems based on inflammation have been developed in order to predict prognosis. Glasgow prognostic scoring, based on C-reactive protein (CRP) and albumin levels, is the most widely known (16). Similarly, pre-treatment NLR is associated with systemic inflammation, and has been shown to have prognostic value and correlate with poor survival outcomes in various cancers.

In malignant diseases, the increase in WBC count is mostly due to cancer-associated myeloproliferation. Cancer cells stimulate myelopoesis and also impair myeloid cell differentiation, thus leading to elevated WBC count. The resulting increase in immature myeloid cells causes immunosuppression of myeloid suppressor cells and consequently triggers tumor progression and metastasis (16).

The relation between cancer and neutrophilia/lymphocytopenia has been demonstrated in various studies (17). Cytotoxic immunity is considered a natural defense mechanism against tumors, and malfunctions in this mechanism can result in disease stage progression and poor prognosis. In patients with advanced cancer, tumor prognosis is strongly correlated with leukocytosis, lymphocytopenia, and CRP (18). In addition, pre-treatment NLR was shown to be associated with prognosis in many cancers. Especially in superficial bladder cancer, there are quite a few studies demonstrating the relation between NLR, recurrence, and progression. (9,11,19)

Consistent with the literature, our results showed that NLR was significantly lower in Ta disease. Furthermore, at NLR lower than 3.22, the probability of disease being stage Ta was found to be 80.6%.

Parameters related to inflammation such as WBC count, NLR, and RDW have been associated with prognosis in many cancers, but to the best of our knowledge, there have been no studies evaluating the combination of these markers in bladder cancer.

RDW is a standard parameter measuring the variability in the size of erythrocytes. It is increased in cardiac diseases in particular but also in systemic inflammation. Rise in RDW can be associated with many types of cancer. In a study by Riedl et al. (12), increase in RDW was associated with poorer overall survival in breast, lung, colon, pancreas, prostate, brain, kidney, and stomach cancers. Likewise, in a study specifically investigating RDW values in colon cancer and colonic polyps, RDW was determined to be significantly higher in colon cancer patients (20).

Although the mechanism is not completely understood, it is believed that RDW is associated with IL-6, tumor necrosis factor, hepcidin, and other cytokines in the circulation which affect the biological behavior of tumor cells (21,22). Moreover, RDW was shown to be associated with poor nutritional status and indicates the nutritional status of patients along with iron, folate, and vitamin B12 (23). A relationship between RDW and IGF-1, the cornerstone of metabolic aging and longevity, has also been reported (24).

In our study, the success rate for determining the Ta group was higher than that for the T1 group. RDW was found to be significantly lower in low-grade superficial bladder tumors. In other words, RDW sensitivity was much higher in Ta superficial bladder tumors compared to T1 tumors. In addition, RDW lower than 15.35 was associated with a 94.4% probability of stage Ta disease. Although the prognostic value of RDW has been demonstrated previously in various cancers, to the best of our knowledge this is the first study to evaluate the utility of RDW in predicting progression in patients with superficial bladder cancer.

Study Limitations

Limitations of the study include being single-center and retrospective and having a small sample size. Larger scale, prospective, multicenter studies will yield more reliable results.

Conclusion

NLR and RDW are basic blood parameters that physicians can easily assess. This study suggests that the combination of NLR and RDW can help clinicians predict lamina propria invasion in superficial bladder tumors.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.B.Ş., C.A., M.M.B., Design: A.B.Ş., M.E., Data Collection or Processing: A.B.Ş., Analysis or Interpretation: A.B.Ş., E.A., Literature Search: A.B.Ş., T.T., Writing: A.B.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Evaluation of Testicular Self-examination Technique and Testis Cancer Knowledge Levels of Final-year Medical Students

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Abstract

Objective: The aim of this study was to evaluate the adequacy of knowledge regarding testicular self-examination (TSE) in final-year medical students and determine the TSE performance rate among male students.

Materials and Methods: This cross-sectional study was conducted with final-year students in the Meram Medical Faculty of Necmettin Erbakan University in the 2015-2016 academic year. The target population of the study was 233 people and all 202 people who agreed to participate in the survey were included. A data collection form consisting of 29 questions was prepared for the study and was completed under observation. Questions regarding TSE technique and knowledge of TSE and testicular cancer were scored as 1 point for each correct answer and 0 points for incorrect answers. Scores ≥6 points for TSE technique and ≥10 points for TSE and testicular cancer knowledge were regarded as adequate.

Results: The mean age of the participants was 24.12±1.32 years; 44.1% were women and 89.6% were single. Nearly 25% of the students reported they knew how to perform TSE and 32.1% had performed TSE before. History of cancer in a first-degree relative was reported by 17.4% of the students, but no students had family history of testicular cancer. Fifty-three of the students who claimed to know how to perform TSE, only 34% (n=18) scored at least 6 points in the TSE technique questions. Evaluation of scores in the TSE and testicular cancer knowledge section showed that 21.3% (n=37) scored above the 10 point limit.

Conclusion: In our study, it was observed that most of the final-year medical students did not have sufficient information on TSE and testicular cancer, and that TSE rates of male students were low.

Keywords: Testicular cancer, testicular self-examination, medical students

Introduction

Cancer is a serious health problem that causes death without early diagnosis and treatment. It accounts for 25% of deaths in developed countries and is the second most common cause after ischemic heart disease among all death causes. Cancer is a life-threatening chronic disease responsible for about 10% of global deaths (1,2,3).

It is well-known that cancer is a difficult disease that affects both the patient and the family physically and emotionally. Despite biomedical developments, cancer is still regarded as synonymous with death, pain, and suffering (4,5). Cancer has many subtypes that can originate from every organ and tissue. One of these is testicular cancer. Testicular cancer is the most common type of cancer in men between the ages of 15-35 (6). It accounts for 23% of the cancers in this age group (7) and approximately 1-2% of all malignant tumors (8,9). Over the past 20 years there has been a 50% increase in incidence (10). It is considered to be an important public health problem in the United States and continental Europe due to the fact that it is the most common cancer between 15 and 35 years of age (11). Cancers are cancers with high treatment success. The main factors in treatment success are early diagnosis, careful grading at the time of diagnosis, appropriate early treatment approach (surgery, chemotherapy, radiotherapy), very close follow-up, and rescue treatment if necessary (12). Although testicular cancer is a rapidly spreading type of cancer, 85-90% of patients recover fully if diagnosed early. For this reason, early diagnosis and treatment of testicular cancer is very important (13). The only way to detect testicular cancer early is to regularly conduct

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a testicular self-examination (TSE), which increases the chance of catching testicular cancer early by nine to ten fold (14). Testicular cancer affects men at a time when they will have important relationships and be faced with family and career decisions (15). In addition to diagnosis and treatment, doctors also have responsibilities such as raising awareness and taking protective health measures for their patients. For this reason, medical students need to acquire the necessary knowledge and skills before graduation. In this study, it was aimed to evaluate whether final-year medical students had sufficient knowledge about TSE and to determine the rate of TSE performance among the male students.

Materials and Methods

This research is a cross-sectional study.

Target Population and Sampling

The study was conducted in final-year students attending the Meram Medical Faculty of Necmettin Erbakan University in the 2015-2016 academic year. During their 6-year medical education, students have theoretical lectures in Term 3, and a two-month pediatric internship in Terms 4 and 6. In Term 5 they have an 8-day pediatric surgery internship and an 11-day urology internship with theoretical and practical lectures about TSE and testicular cancer. The study was conducted on 25 June, 2016. The target population of our research was 233 people. No sample was selected for this study and all 202 persons who agreed to participate in the survey (participation rate: 87%) were included. Our rationale for chosing final-year medical students was: a) They receive courses related to this topic during their training; b) They will soon start their medical practice; c) They are in high socio-cultural group; d) They are an accessible group, and most importantly, e) They come from almost all provinces of Turkey. Thus, we believe the sample is representative of the Turkish population.

Data Collection

A data collection form consisting of 29 questions was developed for this study by scanning the literature. One of the questions is open-ended, while 28 are closed-ended. Seven of the questions are about socio-demographic characteristics, 8 are about the steps of the TSE technique in detail, and 14 assess knowledge level of testicular cancer and TSE (frequency, symptoms and findings, early diagnosis methods). A preliminary data collection form was piloted with 10 people whose data were not included in the analysis of this study. After this pilot study, the final data collection form was written and verbally acknowledged and was completed by all the students at the same time under observation 1 week before graduation. Completion time of the form was approximately 15 minutes.

Dependent variables of the study were having adequate knowledge about TSE, testicular cancer, and TSE technique. Independent variables were age, gender, marital status, history of cancer in first-degree relatives, family history of testicular cancer, reporting knowledge of TSE, and practicing TSE. When evaluating the particpants' responses, each correct answer was worth 1 point and each false answer was given 0 points. According to this scoring system, students could score 0-8 in the section about TSE technique and 0-14 in the section about TSE and testicular cancer. TSE competence was defined as TSE technique scores of 6 and above and TSE and testicular cancer knowledge scores of 10 or above.

Approval for the study was obtained from the Necmettin University Meram Faculty of Medicine Ethics Committee (2016/417, January 22, 2016).

Statistical Analysis

The students' responses were transferred to a computerized database. The data were analyzed using SPSS 24.0 package program. Descriptive statistics for numerical data were mean ± standard deviation (SD), minimum and maximum values and categorical data were expressed in percent (%) distributions. McNemar test was used to determine the relationships between categorical data; t-test in independent groups, double-point correlation (ETA statistic), and Spearman correlation were used to determine the relationships between numerical data. The statistical significance level was accepted as <0.05.

Results

The average age of the students was 24.12±1.32 years. Other characteristics of the final-year students are presented in Table 1. Fifty-three students claimed to know how to perform TSE. When these students were asked about the technique in detail, their mean score was 5.37±1.55 (min:1, max:8). The proportion of students with a score of at least 6 points was 34% (n=18). Six of the students who claimed to know the TSE technique were female, but when asked about the

Table 1. Some characteristics of final-year medical students (Konya/Turkey-2016)

Characteristics	n	%
Sex		
Female	89	44.1
Male	113	55.9
Marital status*		
Single	180	89.6
In relationship (engaged/married)	21	10.5
Cancer in first-degree relatives*		
Yes	35	17.4
No	166	82.6
Testicular cancer in family*		
Yes	0	0
No	200	100
Do you know how to perform TSE?*		
Yes	53	26.6
No	146	73.4
Have you ever performed TSE?**		
Yes	36	32.1
No	76	67.9

TSE: Testicular self-examination, *percentage of respondents, **only male students were asked

technique step-by-step in detail, all of those who answered correctly were male. Only 1 student got a perfect score (8) in the TSE technique section. There was no statistically significant relationship between students who claimed they knew how to perform TSE and those who scored at least 6 points when asked about the technical details (McNemar x2=2.616, p=0.106). The students' mean score in the section about TSE and testicular cancer was 8.03±1.75 (min:4, max:12). Thirty-seven (21.3%) of the students surpassed our predetermined threshold of 10 points. None of the students scored perfectly (14 points) in this section. Relationships between the dependent and independent variables are presented in Table 2 and Table 3. There were no correlations between age and TSE technique score (rho=-0.132, p=0.073), between age and TSE and testicular cancer knowledge score (rho=-0.066, p=0.391), or between TSE technique score and TSE and testicular cancer knowledge score (rho=-0.029, p=0.714).

Table 2. Analysis of the relationships between dependent and independent variables by t-test in independent groups

	T-test in independent groups			
	TSE technique score		TSE and testicular cancer knowledge score	
	Mean ± SD*	p	Mean ± SD*	p
Sex				
Female	4.73±1.51	0.712	8.25±1.79	0.175
Male	4.81±1.40		7.88±1.73	
Marital status				
Single	4.79±1.41	0.898	8.08±1.71	0.369
In relationship (engaged/married)	4.75±1.68		7.70±2.15	
Cancer in first-degree relative				
Yes	4.63±1.45	0.551	7.86±1.72	0.590
No	4.81±1.45		8.06±1.76	
Self-reported knowledge of the TSE technique				
Yes, I know	4.62±1.48	0.304	8.29±1.68	0.239
No, I do not know	4.86±1.40		7.94±1.78	
Self-reported TSE practice**				
Yes, I have	4.89±1.46	0.771	8.31±1.51	0.103
No, I have not	4.77±1.38		7.69±1.79	

TSE: Testicular self-examination, SD: Standard deviation, *arithmetic mean ± standard deviation is presented, **only male students were asked

Table 3. Analysis of relations between dependent and independent variables by double-point correlation (ETA statistic)

	Double-point correlation (ETA statistic)	
	TSE technique score	TSE and testicular cancer knowledge score
	ETA	ETA
Sex	0.013	0.048
Marital status	0.055	0.012
Cancer in first-degree relative	0.036	0.039
Self-reported knowledge of TSE technique	0.046	0.049
Self-reported practice of TSE	0.064	0.018

ETA: European technical approval, TSE: Testicular self-examination

Discussion

In our study, about one-fourth of the students stated that they knew how to perform TSE, but when asked about the examination technique in detail, only one-third of these students really knew the technique. Many students who believed that they knew the TSE technique did not have accurate information. Only 20% of the students in our study had adequate TSE and testicular cancer knowledge scores. These low rates may be interpreted as a deficiency in TSE and testicular cancer lessons, both theoretical and practical, in medical education. These low rates may also be related to the fact that TSE and testicular cancer lessons are not adequately addressed during medical education and that students are not educated and trained at the required levels. Other factors may be that the prevalence of testicular cancer is relatively low and that testicular cancer is not included in the national cancer screening program. In previous studies, knowledge of TSE and testicular cancer and TSE practice rates were found to be low (16,17,18,19,20,21,22). In a study conducted by Bektaş et al. (16) on male nursing students, it was found that 91.8% of the students did not have sufficient knowledge about TSE, 65.6% did not know how to perform TSE, only 11.6% practiced TSE. In a study conducted by Altınel (17) and colleagues in Samsun, 93.8% of the students had never heard of TSE, 3.3% knew how to perform TSE, 76.6% wanted information about TSE, and 18.8% were not able to do TSE correctly. Pour and Çam (18) found that 72.4% of male nursing students were unaware of TSE and 89.4% did not know how to perform TSE. Göçgeldi (19) and colleagues found that only 20.7% of participants had heard of TSE, 8.8% had performed TSE at least once, and 57.6% of those who did not practice TSE did not know the TSE technique. Lechner et al. (20) found that 3% of the participants, Khadra and Oakeshott (21) determined that 28% of their group, and Rudberg et al. (22) found that 5.6% of students had heard of TSE before. We were unable to find another study in the literature in which participants were questioned about the TSE technique in detail as in our study. The other studies evaluated whether the participants did or did not know about TSE based on self-reporting. Therefore, we cannot make a comparison with the literature in this respect. However, it should also be taken into account that there may be a discrepancy between participants' claimed knowledge of the TSE technique and their genuine knowledge of the technique, and rates of those who actually know the technique may be much lower than those specified. In our study, all of the 18 students who knew the TSE technique were male. The lack of women who knew TSE technique may be attributed to their indifference to an examination that they cannot apply in their own bodies. In addition, female students may also prefer not to learn an examination that concerns the male reproductive organs because of social value judgements. Although having a

family member with cancer is expected to raise an individual's sensitivity and awareness of cancer, the results of our study did not support this. Although one-fifth of the students had a first-degree relative with cancer, there was no relationship between family cancer history and dependent variables. This may also be explained by the fact that none of the students' relatives had testicular cancer. In another study conducted in İzmir, there was no relationship between TSE practice and the presence of cancer in the family (16).

Study Limitations

In addition to self-reported knowledge and practice of TSE examination technique, we identified students with genuine knowledge of TSE by asking about the technique step-by-step in detail. We also included both female and male medical students in order to evaluate the knowledge of early diagnosis and examination of a male reproductive cancer. These two aspects distinguish our study from others in the literature. Furthermore, due to the fact that the students were from all provinces and regions of Turkey, our results may be generalized to all of Turkey. While evaluating the results of this study, it is useful to consider some limitations. In this study were unable to identify any factors that might be associated with TSE knowledge and practice.

Conclusion

Most of the final-year medical students in our study did not were not adequately informed about TSE and testicular cancer. It is very important that these students, who are going to be employed as health professionals in the near future, be knowledgeable enough to be able to train their patient groups in TSE. Therefore, the medical curriculum should include detailed lectures on both testicular cancer and TSE that include both practical and theoretical instruction. This training should be evaluated thoroughly after implementation to determine its effectiveness and reshaped according to the results. In addition, we recommend the development of a pre-graduation exam that will serve as a reminder of important topics such as TSE, which provides early recognition of testicular cancer. Conducting similar studies in other medical schools in Turkey will be beneficial.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Necmettin Erbakan University Meram Medical Faculty Ethics Committee (2016/417, January 22, 2016).

Informed Consent:

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: M.U., E.N.Y., Design: M.U., E.N.Y., Data Collection or Processing: M.U., E.N.Y., Analysis or Interpretation: M.U., E.N.Y, T.K.Ş., Literature Search: M.U., E.N.Y, T.K.Ş., Writing: M.U., E.N.Y, T.K.Ş.

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Original Article

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Robotic-assisted Laparoscopic Prostatectomy: Initial Experience of 267 Cases

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Abstract

Objective: To present our experience of 267 consecutive patients treated with robotic-assisted laparoscopic prostatectomy (RALP) and assess the perioperative and postoperative outcomes.

Materials and Methods: We retrospectively analyzed the data of 267 men who underwent RALP in our clinic between March 2015 and April 2018. Preoperative clinical data including age, serum prostate-specific antigen (PSA), biopsy Gleason score, and number of positive cores were noted. Perioperative parameters such as operative time and intraoperative complications were recorded. Postoperative parameters including hematocrit change, length of hospital stay, and catheter removal date were noted. Pathological outcomes included pathological Gleason score; positive surgical margin (PSM) status; extracapsular, lymphovascular, perineural, and seminal vesicle invasion; and lymph node positivity. The Clavien-Dindo system was used to classify surgical complications.

Results: The mean age of the patients was 64.2±6.4 years and the median PSA was 8.27 ng/dL. The mean operative time was 196.4±59.4 min and median hematocrit decrease was 3.9%. The overall PSM rate was 21.34% and this rate increased significantly with final pathological stage from 12.97% for pT2 to 35.48% for pT3 ($p<0.05$). Over a mean follow-up time of 19 months, biochemical recurrence occured in 29 patients (9.7%) and a total of 35 patients (22%) required additional treatment. A total of 29 patients (10.86%) had complications and 1 patient required surgical intervention in the first 48 hours after surgery. The median postoperative hospital stay was 3 days and median time to urethral catheter removal was 10 days.

Conclusion: Our initial experience with RALP is promising. Oncological outcomes were satisfactory, with patients benefiting from the advantages of the minimally invasive surgical approach.

Keywords: Prostate cancer, robotic-assisted laparoscopic prostatectomy, outcomes

Introduction

Prostate cancer (PCa) is the commonest cancer in males in the United States and the second leading cause of cancer deaths (1). In Turkey, it is the second most common cancer in all age groups and affects 11% of men (2). Radical prostatectomy remains the gold standard surgical treatment for localized PCa. Robotic-assisted laparoscopic prostatectomy (RALP) was first reported by Binder et al. (3) in 2000 and became widely used all around the world. RALP has many advantages over open and pure laparoscopic radical prostatectomy. Three-dimensional magnified vision, enhanced ergonomics, and the use of an endo-wrist instrument with seven degrees of freedom in range of motion are the main advantages. High costs, inability to understand tissue or suture tension due to lack of tactile sensation, and collision of robotic arms with each other or the assistant port are disadvantages of this technique (4). In this study, we present our experience with 267 consecutive RALP procedures and assess the perioperative and postoperative outcomes.

Materials and Methods

Data pertaining to 267 men who underwent RALP in our clinic between March 2015 and April 2018 were evaluated retrospectively.

All RALP procedures were performed via transperitoneal approach using 6 trocar ports and a conventional 4-arm da Vinci XI robotic system. We began with initial dissection of the seminal vesicles and the prostate in a posterior fashion,

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then returned to the anterior aspect of the prostate and separated the dorsal vein complex. The neurovascular bundle (NVB) was completely released and the prostate was dissected from the bladder neck. Urethrovesical anastomosis was done continuously using two 15 cm 3-0 V-lock sutures, and an 18-French Foley catheter with 10 mL balloon was inserted. Bilateral pelvic lymphadenectomy (BPLND) was performed in all high-risk and selected intermediate-risk patients according to Briganti et al. (5)'s nomogram. Preoperative clinical data including age, serum prostate-specific antigen (PSA), biopsy Gleason score, and number of positive cores were noted. Perioperative parameters such as operative time, intraoperative complications, and whether BPLND or NVB preservation was done were recorded. Operative time was defined as skin-to-skin time in minutes and includes docking and undocking time. Postoperative parameters including change in hematocrit, length of hospital stay, and time to catheter removal were noted. Pathological outcomes included pathological Gleason score; positive surgical margin (PSM) status; extracapsular, lymphovascular, perineural, and seminal vesicle invasion; and lymph node positivity. The Clavien-Dindo system was used to classify operative complications (6).

Written informed consent forms were obtained from each patient and the study was conducted in accordance with the Declaration of Helsinki. Routinely collected patient data in the database was analyzed retrospectively to evaluate clinical and pathological outcomes. Ethics committee approval was not sought because the study also included retrospective data.

Statistical Analysis

Basic and descriptive statistical analyses were used in this study and all data were expressed as mean or median (minimum, maximum) for numerical variables and as frequencies and percentages for categorical variables. Statistical analyses were done using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY).

Results

The mean age of the patients was 64.2±6.4 years and the median PSA was 8.27 ng/dL (range 0.3-53.4). Preoperative clinical characteristics and perioperative outcomes are shown in Table 1. Skin-to-skin operative time ranged from 174 to 410 min and the median hematocrit decrease was 3.9% (range 0.5-14.5%). The overall PSM rate was 21.34% and this rate increased significantly with final pathological stage from 12.97% for pT2 to 35.48% for pT3 ($p<0.05$). Pathological results and clinical outcomes are shown in Table 2. During a mean follow-up of 19 months, 26 patients (9.7%) received adjuvant radiotherapy to the prostatic fossa due to the biochemical recurrence and 11 patients (4.1%) with lymph node positivity received early adjuvant hormone therapy. Complications are shown in Table 3. A total of 29 patients (10.86%) had complications (each with a single event) and 1 of them (1/267) required surgical intervention in the first 48 hours after surgery due to ileum perforation. Six patients with urethra-vesical anastomosis stenosis and four patients with urethral stricture were treated with endoscopic intervention. The median postoperative hospital stay was 3 days (range 2-7 days) and median time to urethral catheter removal was 10 days (range 10-14).

Table 1. Preoperative clinical characteristics and perioperative outcomes

Variable		Result
Age, years (mean ± SD)		64.2±6.4
PSA, ng/dL median (min-max)		8.27 (0.3-53.4)
Number of cores positive, median (min-max)		3.72 (1-12)
Biopsy Gleason score, n (%)	4-6	172 (64.4)
	7	82 (30.7)
	8-10	13 (4.9)
Risk group, n (%)	Low	137 (51.3)
	Intermediate	94 (35.2)
	High	36 (13.5)
Operating time, minutes (mean ± SD)	-	196.4±59.4
Hematocrit decrease, % median (min-max)	-	3.9 (0.5-14.5)
BPLND, n (%)	-	83 (31.1)
NVB preservation, n (%)	-	53 (19.8)

SD: Standard deviation, PSA: Prostate-specific antigen, BPLND: Bilateral pelvic lymphadenectomy, NVB: Neurovascular bundle, Min: Minimum, Max: Maximum

Table 2. Pathological results and clinical outcomes

Variable		Result
Surgical margin status, n (%)	Positive	210 (78.7)
	Negative	57 (21.3)
Extracapsular invasion, n (%)	Yes	82 (30.7)
	No	185 (69.3)
Lymphovascular invasion, n (%)	Yes	50 (18.7)
	No	217 (81.3)
Perineural invasion, n (%)	Yes	203 (76.0)
	No	64 (24.0)
Seminal vesicle invasion, n (%)	Yes	38 (14.2)
	No	229 (85.8)
Pathological Gleason score, n (%)	4-6	106 (39.7)
	7	141 (52.8)
	8-10	20 (7.5)
Lymph node positivity, n (%)	Yes	11 (13.2)
	No	72 (86.8)
Pathological stage, n (%)	pT2	201 (75.3)
	pT3	66 (24.7)
Biochemical recurrence, n (%)	Yes	26 (9.7)
	No	241 (90.3)
Duration of follow-up, months		19 (3-37)
Additional treatment, n (%)		37 (13.8)

Table 3. Classification of complications using the Clavien-Dindo system			
Complication	**n**	**%**	**Clavien-Dindo grade**
Intraoperative			
Ureteral injury	2	0.74	Grade 4
Ileum perforation	1	0.37	Grade 4
Vascular injury	2	0.74	Grade 4
Tachycardia	1	0.37	Grade 2
Postoperative			
Urethra-vesical anastomosis stenosis	6	2.24	Grade 3
Lymphosel (required drainage)	2	0.74	Grade 3
Lymphosel (not required drainage)	4	1.49	Grade 2
Urine leakage	2	0.74	Grade 2
Urethral stricture	4	1.49	Grade 3
Medical			
Wound infection	2	0.74	Grade 2
Transfusion	3	1.12	Grade 2
Total	**29**	**10.86**	

Discussion

Radical prostatectomy has been a challenging surgery since its introduction in 1905 by Hugh Hampton Young. Due to the deep location of the prostate within the pelvis and its extensive vascularization, radical retropubic prostatectomy has continued to have significant surgical morbidity over the years. The search for less invasive techniques with less blood loss and postoperative pain, shorter hospitalization, and improved quality of life without sacrificing oncological results has led surgeons to learn and perform laparoscopic radical prostatectomy (LRP). Robotic surgery was introduced to overcome the limitations of LRP such as the non-ergonomic instruments, difficulty in urethra-vesical anastomosis and steep learning curve with its three-dimensional magnified vision and endo-wrist instruments.

We reported our first 267 RALP procedures, including the learning curve. It has been suggested that at least 50 cases are needed to gain proficiency in RALP (7,8). Although this study included the initial experience in robotic surgery our operative time was compatible with the literature (9,10). Perioperative mean estimated blood loss was under the average and only two patients required blood transfusion after the operation. None of the patients were converted to open surgery and two major complications that occurred during surgery were treated with robotic surgery intraoperatively. These promising perioperative results may be attributed to the advanced laparoscopic skills of the surgeons before their experience with the robotic system.

The presence of PSM after radical prostatectomy is an independent risk factor for local recurrence and disease progression (11). This factor can be influenced by surgeon experience and the main goal of any urologist should be to reduce the PSM rate. In the current study, the PSM rate was 21.34% and this result was consistent with the literature. In the most extensive literature review, Novara et al. (12) reported a 15% mean rate of PSM in RALP series published between 2008 and 2011 (each including >100 cases), with a range of 6.5-32% and concluded that PSM rate is higher in men with more advanced pathologic stage. Our PSM rates were 12.97% for pT2 tumors and 35.48% for pT3 tumors. Lymph node positivity was 13.2% for patients who underwent BPLND (11/83), which was close to Briganti et al. (5)'s results of 12% (13). Complications are a troubling aspect of surgical interventions and should be handled carefully. We classified complications according to the Clavien-Dindo system in this study and our 10.8% complication rate was within the average range when compared with newer series reporting rates from 5.08% to 19.6% (14,15). We hope to decrease our complication rates as we increase our experience in RALP.

Study Limitations

This study has some limitations. Although it was based on a prospective database the study was retrospective. In addition, the follow-up period was relatively short and oncological outcomes such as biochemical recurrence require further observation. We did not compare our RALP results with outcomes of open or pure LRP in our clinic, which may better demonstrate the advantages of robotic surgery. Lastly, the cohort was small, with 267 cases, and a study with a larger sample size may change the results of some parameters.

Conclusion

Our initial experience with RALP is promising. We were able to transfer our LRP technique to robotic surgery with minimal difficulty. Oncological outcomes were adequate with the patient benefiting the advantages of the minimally invasive surgical approach.

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Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.İ., M.A., M.S., Design: E.İ., Data Collection or Processing: Y.A., H.A., Ö.A., Analysis or Interpretation: Y.A., H.A., Ö.A., Literature Search: E.İ., Y.A., H.A., Ö.A., A.Y., Writing: E.İ.

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Vascular Endothelial Growth Factor and Thrombospondin-1 mRNA Expression in Bladder Tumors: Correlation with Histopathology and Prognosis

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Abstract

Objective: The purpose of this study was to determine genetic expression levels of vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1) in fresh bladder tumor specimens and evaluate their relationship with tumor histopathological features and their prognostic value in recurrence and progression in patients with bladder cancer.

Materials and Methods: Patients who were treated for urothelial cancer of the bladder and followed for at least 6 months were included in this retrospective study. Following RNA isolation from fresh tumor tissue samples recovered from transurethral resection or radical cystectomy specimens, VEGF and TSP-1 mRNA expression was analysed by reverse transcription polymerase chain reaction (RT-PCR). The findings were examined in relation to the histopathological parameters and recurrence and progression rates of the respective tumors.

Results: Sixty-eight patients were included in the study. Mean follow-up time was 22.6 months. In patients with non-muscle-invasive urothelial bladder cancer (NMIBC), rates of recurrence and progression were 64% and 35%, respectively. RT-PCR analyses revealed VEGF mRNA expression in 29 patients (43%) and TSP-1 mRNA expression in 22 patients (32%). Recurrence and progression were observed during follow-up in 64% and 24% of the 25 NIMBC patients with positive VEGF expression, while these rates were 63% and 30% among the 30 NIMBC patients with no VEGF expression, respectively. Rates of recurrence and progression during follow-up were 70% and 30% among NIMBC patients with positive TSP-1 expression and 60% and 26% among patients with no TSP-1 mRNA expression, respectively.

Conclusion: In this study, VEGF and TSP-1 mRNA expression was not associated with histological grade or stage of bladder cancer. There was no difference in VEGF expression in tumor tissues from NIMBC patients with or without disease recurrence. Though lacking statistical significance, a positive correlation between TSP-1 expression and tumor recurrence and progression was seen among the NIMBC patients in our study. Although stimulatory and inhibitory factors are known to regulate angiogenesis, no definitive conclusions have been reached regarding their mechanism of action or the prognostic significance of their up-or down-regulation.

Keywords: Bladder, bladder neoplasms, angiogenesis, tumor markers, molecular markers, prognosis

Introduction

Like other solid cancers, bladder cancer depends on angiogenesis for progressive growth and metastasis (1). Tumors need this neovascularization feature in order to weaken the extracellular matrix and meet their migration and nutrition needs. There are many angiogenesis stimulating and suppressing factors in tumor cells and their microenvironment. During tumorigenesis, the angiogenic phenotype emerges as a result of increased expression of angiogenesis stimulating factors, reduced expression of angiogenesis suppressing factors, or a combination/interaction of both these mechanisms. Folkman (2) described this as the "angiogenic switch" (3). This transformation can cause changes in neoplastic cells ranging from accelerated growth to drug resistance, and even invasive and metastatic capabilities. Thus, exploring the complex process of angiogenesis has potential therapeutic benefits in terms of predicting the biological behavior of tumors and revealing ways to prevent angiogenesis. With the recent development of new molecular techniques, there has been an increase in the number of detailed studies on human cells and tissues at the DNA, RNA, and protein level. Beyond the general histologic structure and DNA content of tumors, advances in molecular biology, immunology, and cytogenetics have enabled us to describe tumors' distinguishing features and biological behavior. The most commonly used method for investigating the role of various tumor markers in the diagnosis

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and prognosis of bladder cancer is immunohistochemistry. Although there are still standardization issues with this method that have not been overcome, it has clinical applications. Other methods such as single-strand conformation polymorphism (SSCP), DNA sequencing, or polymerase chain reaction (PCR)-based investigations are used in experimental research.

Studies on tumor angiogenesis are expected to lead to crucial advances in our ability to distinguish bladder cancer patients at risk of progression and metastasis and identify novel treatment approaches. It is known that tumors that are initially in the same histopathological category can exhibit substantial differences in terms of recurrence and progression over the course of follow-up. Pioneering studies by Chodak and Summerhayes (4) which demonstrated that pieces of bladder tumor can stimulate angiogenesis and that angiogenic factors are present at high levels in the urine of patients with bladder tumors led to further studies on the role of angiogenesis in bladder cancer (5).

In the present study, we investigated gene expression levels of vascular endothelial growth factor (VEGF), one of the main angiogenesis stimulating factors, and thrombospondin-1 (TSP-1), which is an angiogenesis suppressing factor, in tumor tissues obtained from patients with bladder cancer. These genotypic characteristics were compared with classical histopathological parameters used in bladder cancer in order to evaluate the potential role of angiogenic and anti-angiogenic factors in determining recurrence and progression in urothelial bladder cancer.

Materials and Methods

Patients who were treated for bladder cancer and followed for at least 6 months were included in this retrospective study. Archived patient information, pathology reports, and tumor follow-up records were analyzed in terms of patient age, sex, initial tumor grade, initial tumor stage, tumor recurrence, progression of tumor grade and stage, recurrent tumor grade, recurrent tumor stage, and follow-up time (months).

Changes in tumor grade and stage were evaluated using cystoscopy, transurethral biopsy/resection, computed tomography, magnetic resonance imaging, and chest X-ray. Tumor staging was done according to the TNM classification system defined by the Union for International Cancer Control and histological grade between I and III was determined according to the Mostofi system (6). Tumor tissue samples were obtained from transurethral resection or radical cystectomy specimens of patients operated between 1998-2000. Fresh tumor tissues were sent under sterile conditions in dry tissue containers to the Marmara University School of Medicine Department of Urology, Ergun Özalp Research Laboratory and RNA isolation was performed as soon as possible using Trizol®. After isolating RNA from the bladder tissue samples, reverse transcriptase polymerase chain reaction (RT-PCR) was performed using specially synthesized oligonucleotide primers (Table 1). The presence of VEGF and TSP-1 expression in the samples was investigated using RT-PCR analysis. To demonstrate the efficacy of the PCR technique and to prevent false negatives, positive controls were included in each PCR cycle (Access RT-PCR System, Promega, MI, USA). The presence of separate DNA bands for amplification products expected from PCR in agarose gel electrophoresis was regarded as a positive result. The sizes of visualized bands were estimated using a DNA molecular-weight marker (100-bp DNA ladder, Promega, MI, USA) loaded in the last well of the electrophoresis gel. Seeing no bands was considered a negative result. Because the patients had previously consented to the use of their medical data in scientific research provided that their identities were not disclosed, ethics committee approval was obtained as a retrospective study.

Statistical Analysis

Fisher's exact test was used to determine whether there were any non-random associations between the various histopathological features of tumors with and without VEGF and TSP-1 mRNA expression, and chi-square test was used to compare VEGF and TSP-1 expression levels in the NMIBC patient group ("comparison of proportions"). A p value <0.05 was considered statistically significant.

Results

Clinical and Histopathological Evaluation

There were 68 patients in the study group (47 men, 21 women). The mean age of the patients was 63 (41-80) years. Mean follow-up time was 22.6 (8-48) months.

Tumor recurrence was detected during follow-up in 57% (39/68) of all cases. The tumor recurrence rate among patients with non-muscle-invasive urothelial bladder cancer (NMIBC) was 64% (35/55). Of these patients, recurrence occurred in 57% (8/14) of those with initial tumor stage of Ta and 66% (27/41) of those with initial tumor stage of T1. Within the T1 subgroup, recurrence rate was 44% for low-grade tumors and 80% for high-grade tumors. Tumor progression was observed during follow-up in 35% (19/55) of patients with NMIBC. In terms of stage and grade, there were no statistically significant correlation between histopathological characteristics of the bladder tumors and VEGF and TSP-1 mRNA expression (Table 2).

Investigation of VEGF and TSP-1 mRNA Expression Using RT-PCR

VEGF mRNA expression was detected in 29 patients (43%) by RT-PCR (Figure 1). Of these patients, 25 had NMIBC and 4 had

Table 1. Base sequences of oligonucleotide primers synthesized for RT-PCR analysis of genes used in this study (OMIM Genome Database)
VEGF (RT-PCR)
sense primer: 5′ – CGA AGT GGT GAA GTT CAT GGA TG – 3′
antisense primer: 5′ – CCG GAA TTC ACA TTT GTT GTG CTG T – 3′
Thrombospondin (RT-PCR)
sense primer: 5′- CGG GCC GCC GCG CTC CGG TAC ACA C - 3′
antisense primer: 5′- GAG GTC CAG GGT GCC GCC TTG CCA -3′
OMIM: Online Mendelian Inheritance in Man database

Table 2. Distribution of tumors according to histopathological diagnosis in the 68 patients included in the study			
Non-muscle-invasive bladder cancer	**n**	**Muscle-invasive bladder cancer**	**n**
Ta grade 1	9	T2 grade 2	2
Ta grade 2	5	T3 grade 3	9
T1 grade 1	7	T4 grade 3	2
T1 grade 2	20	-	-
T1 grade 3	14	-	-
Low-grade	16	**Low-grade**	_
High-grade	39	**High-grade**	13

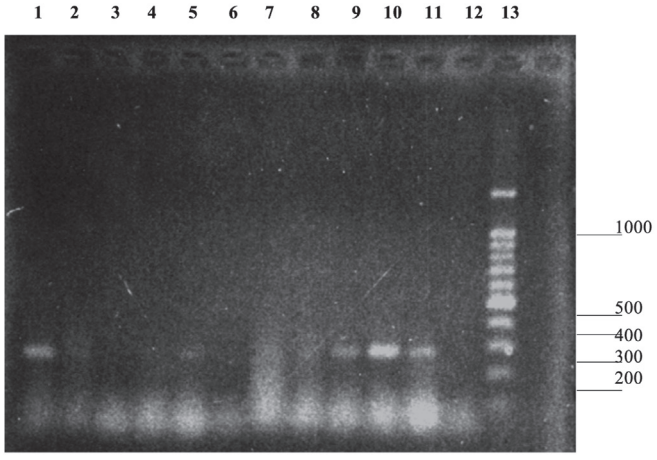

Figure 1. Image of agarose gel electrophoresis of VEGF mRNA RT-PCR products in bladder tumor samples of patient 10. Wells 1, 5, 9-11: patients with positive VEGF mRNA expression; Wells 6 and 12: negative controls; Well 13: 100-bp DNA ladder

VEGF: Vascular endothelial growth factor, RT-PCR: Reverse transcription polymerase chain reaction

MIBC (Table 3). Of the 25 NMIBC patients who were positive for VEGF, tumor recurrence was detected during follow-up in 16 (64%) while progression was detected in only 6 patients (24%). Among the 30 patients with NMIBC who did not have VEGF expression, recurrence rate was 63% (19/30) and progression rate was 30% (9/30) (Table 4). RT-PCR analysis of the tumor samples revealed TSP-1 expression in 22 patients (32%) (Figure 2). Of these patients, 20 had NMIBC and 2 had MIBC (Table 3). Among the NMIBC patients who were positive for TSP-1 expression, the tumor recurrence rate was 70% and tumor progression rate was 30% during follow-up (Table 4). Of the 35 NMIBC patients who tested negative for TSP-1 expression, the tumor recurrence rate was 60% and progression was observed in 26% of the patients.

Discussion

The generation of new blood vessels in tumors is dependent on the equilibrium between angiogenic and anti-angiogenic factors in the environment. As in many solid tumors, the development of angiogenesis is also an important step in the pathogenesis of bladder cancer (7). A strong correlation has been detected between tumoral microvessel density and progressive pathological findings and poor prognosis in patients with bladder cancer (8). Among the various mechanisms that can affect the angiogenic switch in bladder tumors, the most

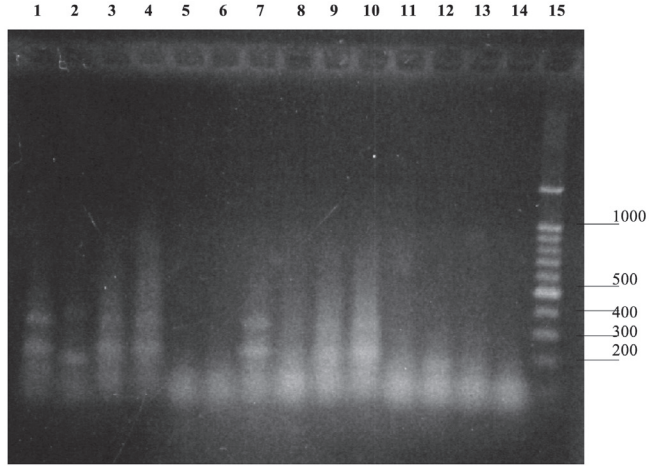

Figure 2. Image of agarose gel electrophoresis of TSP-1 mRNA RT-PCR products in bladder tumor samples of patient 13. Wells 2-4, 7, 10: patients with TSP-1 mRNA expression; Well 12: negative control; Well 15: 100-bp DNA ladder

TSP: Thrombospondin, RT-PCR: Reverse transcription polymerase chain reaction,

Table 3. Histopathologic distribution of tumors with and without VEGF and TSP-1 mRNA expression							
		VEGF (+)	**VEGF (-)**	**p value**	**TSP-1 (+)**	**TSP-1 (-)**	**p value**
Stage	Ta	5	9	-	6	8	-
	T1	20	21	-	14	27	-
	>T2	4	9	-	2	11	-
	NMIBC	25	30	0.37	20	35	0.20
	MIBC	4	9		2	11	
Degree	G1	6	10	-	6	10	-
	G2	15	12	-	11	16	-
	G3	8	17	-	5	20	-
	LG	6	10	0.78	6	10	0.76
	HG	23	29		16	36	

VEGF: Vascular endothelial growth factor, NMIBC: Non-muscle-invasive urothelial bladder cancer, MIBC: Muscle-invasive urothelial bladder cancer, TSP: Thrombospondin, LG: Low grade, HG: High grade

Table 4. VEGF and TSP-1 expression rates in the NMIBC patient group						
	VEGF (+)	**VEGF (-)**	**p value**	**TSP-1 (+)**	**TSP-1 (-)**	**p value**
NMIBC	25	30	-	20	35	-
Recurrence	64% (16/25)	63% (19/30)	0.96	70% (14/20)	60% (21/35)	0.46
Progression	40% (10/25)	30% (9/30)	0.44	30% (6/20)	26% (9/35)	0.73

VEGF: Vascular endothelial growth factor, NMIBC: Non-muscle-invasive urothelial bladder cancer, TSP: Thrombospondin

prominent are overexpression of stimulating factors and/or loss of endogenous suppressive factor production (9). These factors can be produced by tumor cells, secreted from the surrounding extracellular matrix and tumor-related stromal cells, or may be products of inflammatory cells infiltrating the tumor. The most frequently studied stimulants of angiogenesis in bladder cancer are VEGF, thymidine phosphorylase, matrix metalloproteinases (basic fibroblast growth factor b-FGF), carbonic anhydrase 9, and cyclo-oxygenase 2, while the most studied suppressors include angiostatin, endostatin, p53 and thrombospondin-1 (11).

In this study, we investigated mRNA expression levels of VEGF and TSP-1, which are known to have important roles in angiogenesis stimulation and suppression mechanisms, in bladder tumor tissues and its relationship with histopathological classification and prognosis of bladder cancer. In our study, RT-PCR analysis of tumor tissues with histologic features of NMIBC and MIBC obtained from a total 68 patients revealed VEGF expression in 43% of the tumors. The proportion of tumors positive for VEGF expression was higher in patients with NMIBC (45% vs 31%) and in higher grade tumors (44% vs 37.5%). When NMIBC patients were evaluated separately, it was found that tumors with and without VEGF expression had tumor recurrence rates of 64% and 63%, and progression was observed in 40% and 30%, respectively. Comparison of these proportions yielded no statistically significant differences. Campbell et al. (12) observed no significant difference in VEGF levels determined using immunostaining in normal urothelium versus NMIBC and invasive bladder cancer tissues. However, Crew et al. (13) detected higher VEGF concentrations in the urine of bladder cancer patients compared to controls and reported that urine VEGF levels identified using ELISA correlated with recurrence rates in patients with Ta and T1 tumors. Another study of 62 patients with long-term follow-up showed that high initial serum VEGF level had predictive value for overall and cancer-related mortality and could identify high-risk patients who would benefit from preventive treatment (14). However, in another study on 185 patients with Ta/T1 tumor, VEGF expression detected using immunohistochemical method was not associated with bladder cancer recurrence risk or survival (15). In a multivariate analysis of 55 patients with NMIBC who were treated with neoadjuvant MVAC chemotherapy and radical cystectomy, Inoue et al. (16) reported that VEGF expression detected using *in situ* hybridization was an independent prognostic factor for disease recurrence. In studies by O'Brien et al. (17,18), it was reported that VEGF expression was correlated with more aggressive phenotype in the non-invasive tumor subgroup, and that high VEGF expression levels increase the probability of recurrence in low-grade T1 tumors. In our study, RT-PCR analysis of fresh tumor tissues from NMIBC patients revealed no difference in VEGF mRNA positivity between tumors with and without recurrence (46% vs 45%). When evaluated in light of data from the literature cited above, these results suggest that the prognostic value of VEGF expression in bladder cancer has not been clarified to date. There may be several reasons for this. Expression of angiogenesis stimulating factors in bladder cancer can be measured both at the transcriptional and protein level. However, when interpreting results obtained *in vitro*, it must be kept in mind that any contributions from other factors that mediate the angiogenic process or the effects of complex stromal-epithelial interactions and enzymes that cause matrix degradation cannot be taken into account. Furthermore, increased expression (up-regulation) of an angiogenic factor alone is not sufficient for a tumor to become angiogenic. Reduced expression (down-regulation) of certain negative regulators or vascular growth suppressing factors is required. The mechanisms involved in changing the balance between angiogenesis stimulating and suppressing regulators have not been clearly established.

TSP-1 is an extracellular matrix glycoprotein known to be a potent inhibitor of angiogenesis. The role of TSP-1 in tumor angiogenesis and its mechanisms of action are both complex and controversial. In our study, TSP-1 mRNA expression was detected in one-third of all patients. Further analysis of patients with NMIBC showed that recurrence and progression rates were 60% and 26% in patients negative for TSP-1 expression, whereas these rates were 70% and 30% among those positive for TSP-1 expression. In the NMIBC group, TSP-1 mRNA expression was detected in 40% of recurrent tumors and in 30% of tumors that did not recur during follow-up. Although the results of our study were not statistically significant, they suggest that TSP-1 expression may be correlated with tumor recurrence and risk of progression.

In fact, the definitive role of TSP-1 in tumor angiogenesis and progression is a controversial issue: both stimulating and suppressive effects of TSP-1 have been reported in the literature (19-21). In their study on 163 cystectomy specimens, Grossfeld et al. (22) demonstrated using immunohistochemical methods that TSP-1 expression was an independent marker of disease recurrence and overall survival in patients classified according to bladder tumor stage, lymph node status, and histological grade. In addition, it was shown that TSP-1 expression in invasive bladder cancer patients was negatively correlated with p53 expression and microvessel density, and it was suggested that reduced expression of TSP-1, which is a suppressive factor, increases the generation of new vessels in these patients. In line with these findings, Bochner et al. (23) demonstrated that increasing TSP-1 expression in bladder cancer cell cultures resulted in reduced microvessel density and tumor growth arrest. Mutations in oncogenes and tumor-suppressor genes in tumor cells are usually associated with decreased TSP-1 expression. On the other hand, TSP-1 produced by stromal fibroblasts, endothelial cells and immune cells also inhibits tumor progression (24). However, in studies by Qian and Tuszynski (25) it was reported that high levels of TSP-1 mRNA and protein expression in particular have a stimulating effect on invasive tumor biology. The effect of TSP-1 on angiogenesis depends on TSP-1 level, the presence and level of angiogenic stimulants such as bFGF in the tissues, as well as the location of TSP-1 (26). There are various in vitro and in vivo studies in the literature demonstrating that TSP-1 can be negatively correlated with poor prognosis or has no prognostic value (27-29). Our results, although not significant, suggest that TSP-1 expression in NMIBC can indicate poor prognosis. These contradictory observations may be attributable to the complex structure of the TSP-1 protein and its different behaviors specific to various

cell types. The up-regulation of matrix degradation enzymes and inhibitors by TSP-1 may explain both its stimulatory and inhibitory effects. The interaction of signals reaching different tumor and host cells may result in varying response to TSP-1 (30). Discrepancies in the results of these studies demonstrate that there are not yet enough data to elucidate the mechanisms through which TSP-1 expression affects angiogenesis and the biological behavior of tumors.

Inconsistencies in the results of both the current study and other studies in the literature may be related to the limited numbers of patients with various tumor types or to potential methodological flaws such as patient selection criteria, heterogeneous treatment methods, insufficient follow-up time, selection of antibodies used in endothelial staining, the tumor section examined, the researchers' experience, and statistical methods used.

Study Limitations

Limitations of our study include the short mean follow-up time, small patient population, and its retrospective design. Treatment methods used in our heterogeneous patient group might have affected prognosis. Moreover, characteristics of the tumor specimen (peripheral or central sampling of the mass), specimen preparation, and other technical procedures might have affected the results of RT-PCR analyses.

Conclusion

As with other tumors, angiogenesis is profoundly important for the nutrition, growth, invasion, and progression of tumor cells in bladder cancer. The mechanisms of action of the angiogenesis stimulating and suppressing factors involved in the angiogenetic switch, as well as the prognostic value of their up- or down-regulation, have not been definitively determined. However, future studies conducted in bladder tumor tissues may enable identification of tumors with angiogenic phenotype in bladder cancer. In this way, not only will angiogenic factors gain value as prognostic markers, but the mechanisms involved in this process may become targets for novel therapeutic approaches targeting the prevention of cancer progression and metastasis.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: L.T., B.Ö., Design: L.T., B.Ö., Data Collection or Processing: B.Ö., Analysis or Interpretation: B.Ö., L.T., Literature Search: B.Ö., Writing: B.Ö.

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Review

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Intravesical Therapies in Non-muscle Invasive Bladder Tumors

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Abstract

In the European Association of Urology (EAU) 2015 Guidelines for non-muscle invasive bladder tumors, maintenance Bacillus Calmette-Guérin (BCG) therapy is a grade A recommendation. In the intermediate-risk group, re-evaluation is recommended after 1-year full-dose treatment; in the high-risk group, full-dose BCG is recommended for 1-3 years. Intravesical BCG therapy fails in 40% patients in an average of 2 years. In these cases, there is no alternative treatment that is considered effective. In patients with failed BCG, comparison of BCG and gemcitabine showed less recurrence in the long term with gemcitabine while progression and toxicity were similar. Early radical cystectomy should be considered in non-muscle invasive bladder cancer patients with BCG-refractory T1G3 who have good performance status and low comorbidity. In T1 tumors, invade deeper than 3 mm and/or larger than 6 mm in diameter has been associated with a 100% progression rate. BCG decreased recurrence more significantly in high-risk Ta and T1 tumors. In terms of progression of high-risk superficial bladder cancer, comparison of mitomycin C and BCG showed that BCG is superior if maintenance therapy is given. EAU guidelines recommend early bladder chemotherapy instillation (EBCI), in the low-to-intermediate risk group. There was no clear effect of EBCI in the intermediate- and high-risk group. EBCI alone reduces recurrence only in the low-risk group. However, adjuvant intravesical chemotherapy (AIVC) is recommended in the intermediate- and high-risk groups because it improves relapse-free survival. BCG and maintenance BCG therapy were found to be more effective than AIVC in reducing progression and preventing recurrence.

Keywords: Intravesical BCG, intravesical chemotherapy, early bladder chemotherapy instillation, non-muscle invasive bladder tumors

Introduction

Urothelial tumors are the fourth most common of all tumors (1). Bladder tumors account for 90-95% of the cases, while 5-10% originate in the upper urinary tract (2). At initial diagnosis, most cases (75-85%) are non-muscle invasive bladder cancer (NMIBC). According to pathological T staging, 70% are classified as Ta, 20% as T1, and 10% as carcinoma *in situ* (CIS) (3). CIS is prone to muscle invasion (54%) if not treated effectively. Recurrence, progression, and prognosis in NMIBC are predicted using nomograms and European Organization for Research and Treatment of Cancer (EORTC) and Spanish Urological Club for Oncological Treatment (CUETO) classification based on pathological examination of transurethral resection of the bladder (TUR-B) specimens. Adjuvant therapy decisions during follow-up of patients with NMIBC are made based on risk group stratification (low, intermediate, high) according to the clinical and pathological data. The EORTC classification is believed to require strengthening with new parameters due to several reasons such as the absence of additional pathological variants, lack of prognostic factors in the T1 tumor invasion subgroups, and lack of lymphovascular invasion criteria in histopathology (4). Muscle invasion occurs in about 30% of high-risk NMIBCs during follow-up. For high-grade T1 tumors, survival is as low as 34%. This may be attributable to lower grade in initial pathology or the tumor having invasive characteristics (5). A study performed in 2011 showed that patients with invasive progression had worse prognosis than patients whose initial pathology was muscle invasive (3-year survival rate, 37% vs 67%) (6). Previous studies suggested the need to define a "very-high-risk" subgroup within the high-risk NMIBC group in order to avoid the delay of correct treatment (7). As a result, the 2014 European Association of Urology (EAU) NMIBC guidelines included a highest-risk subgroup in addition to the low-, intermediate-, and high-risk groups and recommend early radical cystectomy (Table 1).

In cases with persistent T1 disease after secondary TUR-B, the progression rate for T1G3 patients with 3 poor prognostic factors (age over 70 years, presence of CIS, tumor larger than 3 cm) was reported as 52%. Progression rates of 40% were reported

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for female patients with T1G3 or in the presence of prostatic CIS. Strikingly, invasion deeper than 3 mm and/or greater than 6 mm in diameter was associated with 100% progression in T1 patients (8). The standard follow-up and treatment of NMIBC is cystoscopy. In low-grade Ta disease, 3-year progression is rare (5%), while recurrence rates are high (50-70%). High-grade T1 disease shows substantially high rates of both progression (50%) and recurrence (80%) (9) (Table 2). In this study, we evaluated the clinical role and efficacy of intravesical treatments in NMIBC in light of the studies and reviews conducted to date.

Bacillus Calmette-Guérin Therapy and Prognosis in Bladder Cancer

Bacillus Calmette-Guérin (BCG) vaccine contains attenuated bacteria and its use within the bladder was first described by Morales in 1976 (10). It remains the most effective treatment method for the prevention of recurrence and progression in high-risk NMIBC. Application 2-4 weeks after TUR is recommended to prevent adverse effects. Urinary tract infections must be either excluded or treated before BCG therapy. The procedure should be postponed in cases of traumatic catheterization or other causes of disrupted tissue integrity. After BCG instillation, it should remain in the bladder for 1-2 hours (dwell time). Although this therapy has been used for more four decades, the mechanism of action is thought to be related to the immune response that develops due to local T-cell activation in the bladder mucosa. The local immune response induced by BCG involves both cellular and humoral immune mechanisms. Long-term follow-up of treated patients has revealed predominant increase in T cells and Th1-type cytokines. Local inflammation induced by BCG is called granuloma and is known to play an important role in reducing tumor recurrence (11). The amount of bacteria in standard dose BCG solution should be 108 colony-forming units (CFU)/mL or higher. In their 2013 meta-analysis, Zhu et al. (12) determined that a below-standard BCG dose was associated with higher recurrence rate. However, CUETO previously showed that treatment with a one-third dose reduced recurrence and progression in selected intermediate-risk patients. Although the authors emphasized that full-dose BCG may not be necessary for effective treatment based on their results, their selection of moderate-risk patients for the study makes it difficult to draw a definitive conclusion on this issue (13). Treatment of ureteral and bladder cancer depends on cancer stage and grade. Considering that three-quarters of cases are NMIBC at diagnosis and that one-quarter exhibit progression, there is an undeniable need for additional treatment to prevent both recurrence and progression. BCG is still considered the most effective treatment in the prevention of recurrence and progression. However, careful patient selection is important due to its toxic effects. While intravesical BCG treatment is regarded as unnecessary in the low-risk group, it is strongly recommended for the high-risk group (Table 3). In the intermediate-risk group, the decision to administer intravesical BCG therapy is made based on an evaluation of the likelihood of recurrence and progression. In particular, CIS in addition to superficial bladder cancer should be managed as an invasive tumor. BCG therapy is regarded as first-line treatment for NMIBC patients with CIS (14). In another study it was determined that BCG with maintenance therapy should be the treatment of choice for intermediate- and high-risk patients with CIS (15). Among the recent studies in the literature, a retrospective 2018 study by Yorozuya et al. (16) including 53 patients with variant histology (squamous or glandular differentiation) stands out. Their results support that in NMIBC, intravesical BCG therapy for variant histology has better prognosis in terms of progression and cancer-specific survival than other intravesical treatments (mitomycin C and thiotepa) with no need for additional treatment.

Table 1. Very high-risk subgroup in the European Association of Urology 2014 Guidelines on non-muscle invasive bladder cancer

T1G3 with concurrent bladder CIS
T1G3 with concurrent prostatic urethral CIS
Multiple and/or large (>3 cm) T1G3 tumors and/or recurrent T1G3
Presence of variant histology (especially micropapillary variant)
Lymphovascular invasion
CIS: Carsinoma *in situ*

Table 2. Analysis of 3-year recurrence and progression in low-grade and high-grade T1 tumors (9,36)

Tm pathology	Recurrence (3-year follow-up)	Progression (3-year follow-up)
Low-grade Ta	50-70%	5%
High-grade T1	80%	50%

Bacillus Calmette-Guérin Therapy Doses (Induction and Maintenance)

The currently accepted BCG induction protocol is that described by Morales et al. (10), in which BCG is instilled once a week for six weeks. Intravesical BCG therapy prevents not only prevents recurrence, but also progression. Therefore, it is especially recommended for the high-risk group. Unlike induction, there are no optimal treatment protocols for maintenance therapy. An EORTC study demonstrated the superiority of 3-year full-dose maintenance therapy to 1-year maintenance therapy in high-risk patients. In the same study, this effect was not observed in intermediate-risk patients; therefore, 1-year full-dose maintenance therapy is recommended for the intermediate-risk group. The use of different doses in that study showed no difference between one-third and full-dose maintenance BCG therapy in terms of toxicity (17). Maintenance therapy is a grade A recommendation in the 2015 EAU guidelines. Reevaluation after 1-year full-dose in the intermediate-risk group and continuation of full-dose maintenance BCG for 1-3 years in the high-risk group is recommended (18). Lamm et al.

Table 3. Spanish Urological Oncology Group low- and high-risk groups

Low-risk group	Primary, Ta, solitary, Tm <3 cm, G1, no CIS
High-risk group	T1, G3, presence of CIS
CIS: Carsinoma *in situ*	

(19) analyzed the outcomes of a 6-week induction followed by 3-week treatment cycles performed at 3, 6, 12, 18, 24, 30, and 36 months. Particularly in CIS and selected Ta-T1 patients, maintenance intravesical BCG therapy was shown to approximately double the recurrence-free period.

Adverse Effects of Bacillus Calmette-Guérin Therapy

Local and systemic infections are adverse events that occur after BCG therapy. Hypersensitivity reactions (20) and adverse effects secondary to active infection (21) have been reported. The absolute and relative contraindications to BCG therapy must be known prior to treatment (Table 4) (22). The local adverse effects of intravesical BCG have been investigated in six different randomized studies. The main factors associated with adverse events were age over 70 years, immunosuppression, and urothelial damage. The most common local adverse effects included pollakiuria (71%), cystitis (67%), fever (25%), and hematuria (23%) (23). Lamm (24) reported the rate of severe complications as below 5% in their retrospective study. The most common adverse effect is fever (2.9%), and anti-tuberculosis treatment should be considered if it exceeds 39 °C for more than 48 hours. Severe hematuria (1%), granulomatous prostatitis (0.9%), pneumonia, hepatitis, arthralgia (<0.8%), and more rarely, epididymitis, ureteral obstruction, bladder contracture, and renal abscess have also been reported in the literature. The most common complication is BCG cystitis. It develops within 2-4 hours of treatment and usually resolves within 48 hours. The most feared adverse effect is systemic disease and sepsis. The development of systemic disease has been reported in the first 8-12 weeks. Diagnosis can only be made in the presence of granuloma in tissue biopsies. Infections secondary to BCG therapy may not always manifest with leukocytosis (25). Classical sepsis is very rare (26).

Non-response to Bacillus Calmette-Guérin

Intravesical BCG therapy fails in 40% of NMIBCs in an average of 2 years. In such cases, there is no alternative intravesical therapeutic agent considered completely effective. Progression is rare in patients with initial low-grade pathologic diagnosis. For NMIBC patients classified as high-risk, the probability of muscle invasion and progression must not be overlooked. The main factors in treatment failure are previous exposure to mycobacteria, inappropriate BCG dose, lack of expected cellular immune response in the individual, and therapies initiated based on missing or inaccurate staging (27). Early cystectomy is recommended after failed BCG therapy in T1G3 patients with good performance status and low comorbidity. Promising studies on the development of bladder-sparing intravesical therapies for patients with treatment failure are ongoing.

Table 4. Absolute and relative contraindications for BCG therapy

Absolute contraindications for BCG	Relative contraindications for BCG
- Immunosuppression - History of BCG sepsis - Macroscopic hematuria - Total incontinence - Early post-TUR - Traumatic catheterization	- Urinary tract infection - Liver disease - History of tuberculosis - Advanced age - Poor performance status

BCG: Bacillus Calmette–Guérin, TUR: Transurethral resection

Intravesical Chemotherapy in Non-muscle Invasive Bladder Cancer

The addition of IVCT to TUR-B aims to eliminate residual tumor and prevent progression and recurrence (28). The superiority of any of the IVCT agents (mitomycin C, epirubicin, pirarubicin, and thiotepa) to one another has yet to be demonstrated (29). Choice of drug is based on cost, adverse effects, and practitioner experience. Mitomycin C is the most frequently used agent. Its mechanism of action is the inhibition of DNA synthesis. It is an antimicrobial and anticancer agent obtained from *Streptomyces* species. In normal conditions, systemic absorption is limited due to its molecular weight (334 kD). However, it is not recommended for patients with mucosal damage, widespread resection, and radiotherapy (7). Although an optimum protocol has not been established, urine alkalinization followed by instillation of 40 mg/20 mL mitomycin C and dwell time of 1 hour was recommended (30). When applied as 20 mg/50 mL dose, recurrence was reported to decrease from 57% to 17%, and when used for 6-8 weeks at higher doses (40-80 mg), the proportion of high-grade bladder cancers that were recurrence-free at 2 years was reported as 75% (31). Applying mitomycin C with hyperthermia (41-44 °C) was reported to increase treatment efficacy and survival. However, the use of this technique is limited due to increased cost associated with the special catheter necessary for hyperthermia, increased adverse effects of mitomycin C due to hyperthermia, and patient compliance problems (32). The most common effects associated with mitomycin C toxicity are chemical cystitis (40%) (33) and allergic skin reactions (5-12%) (34). In intravesical applications performed after TUR-B, normal epithelialization of healing areas may be disrupted and replaced by dystrophic calcification, or extravasation from the bladder may cause necrosis, peritonitis, and pelvic pain (35). A 2003 review by Shelley et al. (36) including 6 studies compared BCG and mitomycin C treatments in Ta and T1 tumors. BCG resulted in a more pronounced reduction in the recurrence of high-risk Ta and T1 tumors. Local adverse effects (dysuria, pollakiuria, cystitis, hematuria) and systemic adverse effects (fever, fatigue) were in favor of BCG. A total of 108 patients were divided into passive mitomycin C, electromotive mitomycin C, and BCG groups. Complete response rates at 6 months were 31-58% for passive and electromotive mitomycin C, respectively, and 64% with BCG. Adverse effects were most frequent in the electromotive mitomycin C group and least frequent in the group administered BCG only (37). Five studies with an average follow-up of 26 months compared BCG with and without maintenance therapy to mitomycin C in terms of progression of high-risk superficial bladder cancer. As a result, it was emphasized that BCG treatment is superior if maintenance therapy is provided (38). In another study, 212 T1 patients were divided into two groups, BCG only or sequential BCG and electromotive mitomycin C. Both groups underwent maintenance therapy. At the end of 88 months of follow-up, all parameters (recurrence, progression, and survival) were better in the group that received sequential treatment. Disease-free interval was 69 vs 21 months, recurrence rate was 42% vs 58%, and disease-specific mortality rate was 6% vs 16%. In terms of preventing progression, the difference in progression rates was

noteworthy (16.2% with BCG, 9.3% with sequential treatment) (39). In their meta-analysis of the long-term outcomes of a total of 2820 patients in 9 studies, Malmström et al. (40) compared mitomycin C and BCG therapy. Over 4.4 years of follow-up, the recurrence rate was 43%. There was no difference between BCG and mitomycin C in terms of time to first recurrence. If maintenance BCG was administered, recurrence was 32% lower compared to mitomycin C. A notable recent publication is a 2018 review by Chantada-Abal et al. (41) which evaluated the safety and efficacy of sequential intravesical BCG and mitomycin C in NMIBC. Their analysis revealed that sequential treatment does not cause more toxicity and leads to prolonged disease-free survival by reducing tumor progression more than BCG or mitomycin C monotherapy. They emphasized the need for further clinical research to enable the adoption of combination therapy into routine practice. Anthracyclines (valrubicin, epirubicin, doxorubicin): drugs in this group have lower systemic absorption and cause fewer systemic adverse effects due to their higher molecular weight. They exert their effects by inhibiting DNA topoisomerase 2 and protein synthesis, and via free radical formation and direct cytotoxicity on the cell membrane (42). Valrubicin is FDA-approved for BCG-refractory CIS patients who are ineligible for cystectomy. A complete response rate of 21% was reported in 90 BCG-refractory CIS patients. Adverse effects are local (symptoms of bladder irritation such as cystitis and hematuria) (43). Epirubicin has fewer adverse effects than doxorubicin but similar efficacy (31). Epirubicin is used at various doses between 20-100 mg, but is most frequently used at 50 mg/50 mL once a week for a total of 8 weeks. In a study by EORTC, comparison of single-dose 80 mg epirubicin with TUR-B alone showed that epirubicin reduced recurrence by 12-15% (44). More recently, intravesical BCG and epirubicin therapies in patients with Ta and T1 bladder cancer were analyzed in terms of recurrence, progression by stage, mortality, distant metastases, and adverse effects. Five studies (549 patients treated with BCG and 562 with epirubicin) were included in the analysis. Recurrence rate was 51.4% in the epirubicin group and 35.5% in the BCG group. Differences between the two groups in terms of distant metastases and progression were not statistically significant. In 2 of the 5 studies analyzed, mortality and morbidity were equal in both groups. Local (cystitis, hematuria) and systemic toxicity (fever, fatigue) were more common in the BCG group (45). Gemcitabine acts by inhibiting cellular growth and inducing apoptosis. It has proven systemic efficacy in metastatic bladder cancer. While the results of its intravesical use are promising, more phase 3 studies are required to determine dosing and efficacy. In primary Ta-T1, patients who did not have CIS and did not receive additional treatment had comparable recurrence and progression rates while adverse effects were less common than with BCG (10% vs 45%). In the high-risk NMIBC group, compared to BCG alone, gemcitabine therapy was associated with shorter time to recurrence (25.5 months vs 39.4 months) and higher recurrence rate (53% vs 28%). In BCG-refractory patients, comparison of BCG and gemcitabine showed that recurrence occurred later and less frequently (52% to 87%) with gemcitabine therapy. Progression and toxicity were similar (46). Doxatel is an antineoplastic agent that acts by disrupting the microtubular network during cell division. In 2013, 54 BCG non-responders were followed for an average of 39.1 months. Complete response was achieved in 59% of the patients. Recurrence-free survival was 40% in at 1 year and 25% at 3 years. Cystectomy was performed on 24% of the patients. The 5-year disease-free survival rate was reported as 85% (47). Thiotepa is an FDA-approved agent and was the first to be used intravesically in NMIBC. However, it caused severe adverse effects because it is readily absorbed through the bladder mucosa and passes into the systemic circulation due to its low molecular weight. It is hardly ever used today due to its systemic (myelosuppression and secondary leukemia in up to 54%) and local (irritative urinary symptoms up to 70%) adverse effects (48). Interferon alpha is an immunomodulatory and antiproliferative agent. In terms of recurrence, it is less effective compared with BCG and mitomycin C (49).

Early Single-dose Intravesical Chemotherapy

One of the mechanisms regarded as most important in tumor recurrence is the implantation of residual tumor cells in the bladder after TUR on the mucosal surface. The purpose of early single-dose IVCT is the chemoresection of free tumoral tissues. Technically, it is recommended within the first 24 hours (preferably within the first hours) after TUR-B (50). It is listed as a recommendation in the EAU guidelines for the treatment of low-to-intermediate risk bladder cancers. In a meta-analysis of 1476 patients by Sylvester et al. (51), it was shown that recurrence decreased by 11.7% with early single-dose IVCT. Independent of agent used, recurrence rates are reported as 65% in patients with multiple tumors and 36% in patients with solitary tumors. In a study evaluated based on the EORTC bladder cancer risk classification, it was shown that epirubicin treatment reduced recurrence by 28% in the low-risk group (score 0-2) but had no effect on recurrence in the high-risk group (score 3 or higher). It was emphasized that the best response to treatment was in cases with solitary tumors, and that the same response was not achieved in recurrent multifocal cases (52). Solsona et al. (53) reported that early single-dose IVCT only prevents recurrences occurring within 2 years after TUR-B, but did not prevent recurrences in the longer term. No clear effect of early single-dose IVCT could be demonstrated on progression, recurrence, and time to recurrence in the intermediate- and high-risk group. As a result, the EAU guidelines recommend early single-dose IVCT for low- to intermediate-risk patients with a single tumor focus (54). Choice of drug is based on past experience, accessibility, low cost, and fewer adverse effects. There are no drugs with definitively proven superiority. It is not recommended for wide and very deep resections due to the possibility of extravasation from the bladder and associated risk of systemic toxicity. Secondly, the most important hidden factor in treatment success may be a good pathological examination and obtaining a second confirmation from a different pathology department. Based on these analyses, intravesical approaches to NMIBC can be summarized as follows: early single-dose IVCT alone decreases recurrence only in the low-risk group, and adjuvant IVCT is recommended in the intermediate- and high-risk groups because it increases recurrence-free survival (55). Although the duration of adjuvant IVCT is controversial, less than a year is recommended (56). When TUR-B + IVCT

was compared with TUR-B alone, the recurrence rate decreased by 13-14% with the addition of IVCT (57). BCG and BCG maintenance therapy were found to be more effective than IVCT in reducing progression and preventing recurrence (58).

Ethics

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Review

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The Diagnostic and Prognostic Significance of MicroRNA-21 in Non-muscle Invasive Bladder Tumors

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Abstract

Bladder cancer (BC) is one of the commonly diagnosed urological cancers that causes human death, ranked as the seventh most common cancer worldwide. To date, no reliable diagnostic tool has been defined to recognize non-muscle invasive bladder tumors other than cystoscopy. For this reason, scientists have focused on finding new non-invasive biomarkers that can be used to diagnose BC with higher specificity and sensitivity. The purpose of this study was to evaluate the diagnostic role and prognostic significance of microRNA-21 (miR-21) in non-muscle invasive bladder tumors. In this review, the overall diagnostic performance of miR-21 was discussed on non-invasive BC based on a literature search of PubMed and Cochrane Library. Although findings are insufficient, promising results have been reported regarding circulating miR-21 as a biomarker for BC prospective studies with larger numbers of participants are needed.

Keywords: Non-muscle invasive bladder tumor, microRNA-21, diagnostic marker

Introduction

Bladder cancer (BC) is one of the urological cancers that causes the most deaths worldwide. It is the seventh most commonly diagnosed cancer (1). In 2016, more than 76,000 newly diagnosed cases of BC were reported in the United States and more than 16,000 of those resulted in death (2). BC is 3-4 times more common in men than in women (3). There are geographic variations in the incidence of BC and smoking has an important role in its etiology (4).

BC is classified into two main groups, non-muscle invasive BC (NMIBC) and muscle-invasive BC (MIBC), based on its pathologic and clinical features (5). Approximately 70% of cases are NMIBC at initial diagnosis, and the 5-year survival rate after endoscopic resection of these tumors is around 80% (6,7,8). In 50-70% of NMIBC cases, recurrence is observed in the first two years, while 10-20% of cases progress to MIBC, in which the chance of 5-year survival decreases to 56% (9,10). Although it does not require life-long follow-up, patients diagnosed with BC face invasive procedures like cystoscopy, as well as associated complications such as infections and trauma, due to the frequency of recurrence in the first two years. Progression to MIBC is detected in 20-30% of patients with high-risk superficial BC treated with transurethral resection (TUR) followed by intravesical Bacillus Calmette-Guérin (BCG) therapy (11). Early detection of progression is important because the mortality rate is as high as 44% in MIBC, which can progress rapidly and metastasize despite the availability of effective treatment strategies (8,9,12,13). Although various diagnostic tools and biological markers have been developed to predict the recurrence and progression of NMIBC, most have been shown to have inadequate efficacy and accuracy due to the heterogeneous nature of BC (14,15,16). BC is diagnosed by cystoscopy and urine cytology as well as tumor markers like nuclear matrix protein-22 (NMP-22) and bladder tumor antigen (BTA). The most sensitive of these diagnostic methods is the combination of cystoscopy and urine cytology (17,18). Although cystoscopy is regarded as the gold standard method for the diagnosis of BC, its main disadvantages are that it is invasive, causes patient discomfort, and must be performed by a urologist (19). While urine cytology is a non-invasive method, it is generally more successful in the diagnosis of high-grade and high-stage BCs compared to low-grade tumors (20). NMP-22 and BTA are currently used as urinary biomarkers and are simple, rapid, and non-invasive tests for BC screening. NMP-22 and BTA have specificity of 47-100% and 29-83% and sensitivity of 55-98% and 56-86%, respectively, and therefore they cannot be recommended as ideal diagnostic methods (21,22,23). Non-invasive biomarkers with higher sensitivity and specificity are needed to enable earlier detection of BC. For this reason, new biomarkers should be identified that can be used alone or in combination with parameters affecting BC prognosis in order to identify patients with poor prognosis in advance. MicroRNAs (miRNA) are single-stranded, non-coding RNA gene products, usually 22 nucleotides in length, that are involved in the regulation of gene expression (24). Recent studies suggest that abnormal miRNA structures are associated with the development, progression, and prognosis of various human

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cancers (25,26). Non-invasive biomarkers for the diagnosis of BC can be developed from urinary or circulating miRNAs (27,28,29,30,31,32,33,34).

In this study, we address the diagnostic value and prognostic significance of miRNA-21 in NMIBC.

Discussion

MicroRNA molecules are single-stranded, non-coding gene products 22 nucleotides in length whose roles in human disease are being investigated (35). These molecules post-transcriptionally regulate gene expression by binding to the 3'-UTR region of target mRNAs, resulting in the degradation or translational inhibition of the target mRNA (36). After these molecules that regulate gene expression and various biological processes were first identified in 1993, they were later reported to also affect proliferation, apoptosis, metabolism, and immune mechanisms. The up- or down-regulation of miRNA has been established as a biomarker in numerous cancer types and, accordingly, is thought to have potential utility in the diagnosis or prognosis of various cancers of colorectal (37), breast (38), lung (39), and ovarian (40) origin (25,26,41,42). It has been shown that bladder tumors, like other known solid tumors, also contain hypoxic regions and that excessive release of hypoxia-inducible markers is associated with poor prognosis (43). In 2015, Blick et al. (44) demonstrated that miRNA-210, miRNA-193b, miRNA-145, miRNA-125-3p, miRNA-708, and miRNA-517 were associated with hypoxia in BC cells. The same study demonstrated the functional significance of hypoxia-induced miRNAs and showed that miRNA-145 controlled BC cell apoptosis. miRNA-21 was shown to have a p53-mediated anti-apoptotic effect by specifically targeting programmed cell death mRNAs (45,46). A study conducted by Liu et al. (47) in 2011 showed that the overexpression of miRNA-21 in a prostate cancer cell line increased the release of hypoxia-induced factor 1alpha (HIF-1a) and vascular endothelial growth factor.

miRNA studies on the diagnosis and prognosis of BC showed that these molecules can be obtained from urine (27-34). Although the patient's age and sex or the presence of hematuria may adversely affect the biomarker quality of miRNA, detection of miRNA in urine should be considered an important finding in terms of BC. In recent years, studies have been published on the prognostic significance of different miRNA molecules in patients with BC. In 2016, Zhang et al. (48) reported that miRNA-155 analysis in cell-free urine samples of NMIBC patients had diagnostic value with 85.8% sensitivity. In another study investigating the role of miRNA-203 in predicting treatment response in patients planned to start cisplatin-based chemotherapy for BC, it was reported that low miRNA-203 level predicted progression and poor prognosis while overexpression of miRNA-203 may increase the sensitivity to cisplatin by directly stimulating apoptosis (49). It was also reported that miRNA-214 down-regulates oncogenic P53 and DNA damage-regulated gene 1 and that this is a determining factor in BC prognosis (50). In 2010, Kiemeney et al. (51) expanded their earlier study on Dutch and Icelandic populations to include some European countries, increasing the number of patients to 4,739 and the number of controls to 45,549, and examined the relationship between DNA variants and BC using the 20 best known markers. In this study, they demonstrated that the T allele of rs798766 on 4p16.3 was associated with low-grade and noninvasive BC (51).

miRNA-21, which is considered an oncogene, is frequently upregulated in BC patients and supports tumor cell proliferation and metastasis by interfering with tumor suppressor checkpoints (52,53). Although it is believed that miRNA-21 may have utility as a diagnostic and prognostic biomarker because of its increased release in certain cancers, there are conflicting reports concerning its diagnostic power and prognostic value. One of these was a meta-analysis by Wang et al. (54) in which a total of 528 studies were reviewed and the results of 17 studies that met the study criteria were used to evaluate the relationship between miRNA-21 levels and survival in patients with cancer. Eleven of these studies focused on the diagnostic value of miRNA-21 and 9 studies evaluated prognosis, and plasma miRNA-21 was determined to have sensitivity and specificity of 75.7% and 79.3%, respectively, when used as a diagnostic biomarker. Because coagulation could affect miRNA-21 expression, serum miRNA-21 measurements were also evaluated but no difference was found in terms of sensitivity and specificity (54). A limitation of this meta-analysis was that it did not include studies investigating the relationship between miRNA-21 and BC. In a study examining the relationship between bile duct cancers and miRNA-21, Kishimoto et al. (55) reported its negative predictive value (NPV) as 76.6%. Kotb et al. (56) reported this rate as 90% for prostate cancer. In a study investigating response to preoperative chemoradiotherapy in locally advanced rectal cancers, the NPV for miRNA-21 was found to be 42.8% (57). While it is not surprising that the NPV of miRNA-21 varied for different cancer types in these studies, there is currently no ongoing or completed study showing the NPV of miRNA-21 in BC. In a 2015 study by Zhang et al. (58), TUR was performed on 53 patients with BC who had not received neoadjuvant therapy and RNA was extracted from the specimens. They formed a control group by obtaining healthy bladder tissue from patients who underwent TUR-prostatectomy (TUR-P) due to benign prostate hyperplasia. Patient characteristics such as age, tumor grade, tumor number, stage, and size, recurrence rates, and lymph node involvement were recorded. They determined that miRNA-21 expression was upregulated in BC tissues compared to normal bladder tissues. Upregulation of miRNA-21 was associated with tumor stage, grade, and lymph node metastasis, but not with patient sex, age, tumor size, number of tumors, or recurrence. However, the authors cited the small patient number as a limitation of the study and acknowledged that more extensive studies are needed (59).

Mitash et al. (60) conducted another study with a limited number of cases and short follow-up period, in which they reported that increased miRNA-21 expression was associated with recurrence in NMIBC and that miRNA-21 level was negatively correlated with time to recurrence. It has long been known that high-grade T1 NMIBC is prone to recurrence. The epithelial-mesenchymal transition (EMT) promotes tumor cell proliferation, invasion, and migration, thereby increasing tumor aggressiveness. Histopathologic findings of vimentin

upregulation and e-cadherin down-regulation, which are indirect indicators of EMT (61), plus miRNA-21 overexpression also indicates the tumor will undergo an aggressive transformation (62,63). In a study investigating the critical role of miRNA-21 in cell proliferation, apoptosis, and chemosensitivity in addition to its oncogenic role in BC, it was reported that this molecule will facilitate the differential diagnosis between NMIBC and MIBC. With that study, the authors reported for the first time that increased miRNA-21 level conferred chemoresistance against doxorubicin, an agent used in the treatment of BC, through different mechanisms by stimulating down-regulation in the T24 cell line and that it carried the potential for developing treatment strategies for BC in the future (64).

Conclusion

The results of the few studies demonstrating the importance of miRNA-21 in monitoring BC and predicting prognosis are promising despite some limitations. Extensive prospective studies with larger patient numbers are needed to determine the utility of miRNA-21 as a biomarker.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: N.A.M., Ö.Ç., Design: N.A.M., Ö.Ç., Data Collection and Processing: Ö.Ç., Analysis and Interpretation: N.A.M., Ö.Ç., Literature Search: Ö.Ç., Writing: Ö.Ç.

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Review

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Prostate-specific Membrane Antigen-Based Nanomedicine Applications in the Diagnosis and Treatment of Prostate Cancer

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Abstract

Nanomedicine is a branch of nanotechnology that includes the development of nanostructures and nanoanalytical systems for various medical applications. The rapid development of nanomedicine offers new possibilities in cancer diagnosis and treatment. New therapeutic strategies in cancer research using nanoparticles are being developed in order to improve the specificity and efficacy of drug delivery, thus reaching maximal effectiveness with minimal side effects. Due to its selective overexpression in prostate cancer (PCa), prostate-specific membrane antigen (PSMA) has been recognized as a highly promising target for diagnostic and therapeutic applications. This review provides an update on the PSMA-based nanomedicine applications in PCa.

Keywords: Prostate cancer, PSMA, nanomedicine, nanotechnology, theranostic concepts

Introduction

Theranostic Concept

Targeted cancer therapy can improve progression-free and overall survival. However, the greatest barrier to targeted therapy is identifying the patients who will benefit from it. Therefore, predictive markers are urgently needed. Radiolabeled probes can be used as predictive markers. For instance, target expression can be confirmed by positron-emitting tomography (PET) using F-18- or Ga-68-labeled ligands. The same ligands can be labeled with therapeutic radionuclides (Lu-177/Y-90) for radioligand therapy (RLT). This combination is called a theranostic pair.

Nuclear medicine specialists used this method with I-131 to treat metastatic thyroid adenocarcinoma in 1946 (1). By using I-131 in diagnostic screening, they were able to identify patients with differentiated thyroid carcinoma who would benefit from treatment with a higher dose of I-131 (2). In the 1990s, the theranostic approach progressed toward neuroendocrine tumors (NET). Somatostatin analogs DOTATOC, DOTANOC, and DOTATE were developed to selectively bind to NETs overexpressing somatostatin receptors (SSTR) (3,4,5). Labeling these SSTR agonists with different radionuclides enabled the combination of diagnostic imaging (Ga-68) with radioligand treatment (Lu-177/Y-90) (3,4,5). NETTER-1 was the first randomized prospective study comparing Lu-177-labeled DOTATE with octreotide long-acting repeatable (LAR) in metastatic NET. Lu-DOTATE RLT significantly prolonged progression-free survival (PFS) compared to octreotide LAR (6). Progression was 4.8-fold more frequent among patients who received high-dose octreotide than in those given Lu-177DOTATE (HR: 0.21, 95% CI: 0.13-0.34) (6). This treatment is currently known as peptide receptor radionuclide therapy (PRRT) and is widely accepted and used in patients with NET (5).

Prostate-specific Membrane Antigen as a Target in Prostate Cancer

Prostate-specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II, is a promising theranostic target (7-9). PSMA is a type II transmembrane protein comprised of a small intracellular segment, a transmembrane domain, and an extracellular domain containing the catalytic site (8,9). PSMA is expressed at low levels in various tissues such as prostate, brain, small intestine, and kidney (8,9). While PSMA has different enzymatic functions in brain and small intestine, its enzymatic function in the prostate is not yet clear (9). Most importantly, PSMA is overexpressed in prostate cancer cells and its expression

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level is correlated with pathological grade (10,11,12,13,14,15). Moreover, after ligand binding, PSMA is internalized via clathrin-coated pits and endocytosis (11,16). These characteristics have led to the development of therapeutic PSMA ligands labeled with different radionuclides.

PSMA-targeted Agents

One of the first probes that targeted PSMA was Indium-111 capromab pendetide (ProstaScint®). Capromab is a monoclonal antibody that binds to the cytoplasmic domain of PSMA (16). Probes that target the extracellular domain of PSMA, such as antibody J591, have been developed to improve tumor uptake. However, antibody-based approaches have limited diagnostic potential (8). In recent years, nanoparticle PSMA ligands have been developed. One of these is Ga-68-PSMA-11, which is the most commonly used PET probe in PSMA-based imaging (17). Ga-68-PSMA-11 shows excellent biodistribution and high tumor uptake (18). However, PSMA-11 cannot be labeled with Lu-177 or Y-90 for RLT (19). Therefore, nanoparticle ligands with different chelators were developed. Weineisen et al. (19,20) synthesized DOTAGA-FFK (Sub-KuE), which can be labeled with Ga-68 or Lu-177/Y-90, and its optimized version, PSMA I&T. PSMA I&T has good dosimetry and similar biodistribution to Ga-68-PSMA-11 (20). A research group in Heidelberg also developed PSMA-617, which can be labeled with Ga-68 or Lu-177/Y-90. This probe showed high affinity to PSMA and high tumor base uptake (21,22). Based on their similar biodistribution, PSMA-11 and PSMA-617 are frequently used in combination as diagnostic and therapeutic agents (22). Moreover, commercial ligands labeled with Tc-99m or I-131 have been developed as theranostic pairs (23,24). Mease et al. (25) and Cho et al. (26) synthesized 18-FDCFBC, which is an F-18-labeled small-molecule PSMA inhibitor. This agent reliably detects prostate cancer. The second-generation probe 18-FDCFPyL has shown higher affinity for PSMA and tumor uptake than 18-FDCFBC (27). This probe has good dosimetry and biodistribution, but cannot be labeled with Lu-177 or Y-90 for RLT (28).

Here we present an overview of PSMA-targeted diagnosis and RLT.

PSMA Imaging for Primary Diagnosis

Imaging serves two purposes in the primary diagnosis of prostate cancer. The first is to detect disease progression in patients who have biopsy-proven disease or high metastasis risk, and the second is to determine primary tumor location in patients with high suspicion but negative biopsy (29). Magnetic resonance imaging (MRI) is currently the preferred modality for T staging (29). T2-weighted, dynamic contrast-enhanced, and diffusion-weighted sequences are used to identify tumor involvement, extracapsular extension, seminal vesicle invasion, and/or other organ involvement. Moreover, combining these protocols with multiparametric MRI (MP-MRI) enables the differentiation of benign and malignant prostate tissue (30). MRI is superior to C-11-choline PET/CT (31,32,33), FDG-18 PET/CT (31), and ultrasound-guided biopsy (34) for primary diagnosis.

PSMA PET Imaging for T Staging

Rowe et al. (35) reported that MRI had higher sensitivity in the detection of primary lesions compared to F-18-DCFBC PET/CT. The importance of the second-generation radionuclide tracer F-18-DCFPyL in the detection of primary prostate lesions has not yet been evaluated. Ga-68-PSMA-11 PET is superior to MP-MRI, with a sensitivity of 49-76% in different populations (36,37). Ga-68-PSMA-11 uptake was found to be significantly higher in histopathology-positive areas than negative areas (37). The accuracy of GA-68-PSMA-11 PET/CT in the detection of seminal vesicle invasion and extracapsular tumor spread was 86% and 71%, respectively (37). Based on these findings, PSMA imaging has the potential to replace MP-MRI for determining tumor location.

Clinically, treatment options for localized prostate cancer may vary from active surveillance to radiotherapy and radical prostatectomy. T staging is important for determining the best approach. European Association of Urology guidelines recommend MP-MRI for T staging (29), and although PSMA is superior in the detection of primary prostatic lesions, increased diagnostic accuracy has not been shown to have a significant effect on patient management. Of 15 patients who underwent MRI and were diagnosed with prostate cancer, planned radiotherapy was changed in 26.4% after additional PSMA imaging (additional dose, wide area) (38). However, due to inadequate long-term follow up and lack of a control group, the effects of these findings on patient outcomes are not known. Therefore, further studies are required to determine whether PSMA PET influences clinical management at initial diagnosis of patients with prostate cancer.

PSMA Imaging for N Staging

Many studies have demonstrated high reliability of Ga-68-PSMA-11 PET/CT in N-staging at primary diagnosis. Budäus et al. (39) retrospectively compared lymph node findings in preoperative Ga-68-PSMA-11 PET/CT with histopathology in 12 patients and reported a low detection rate of 33.3% and mean sizes of detectable and undetectable lymph nodes of 13.6 mm and 4.3 mm, respectively. Later studies showed that Ga-68-PSMA-11 PET/CT or PET/MRI were superior to conventional imaging techniques in the detection of lymph nodes (40,41,42). In one study, lymph node metastasis was detected using Ga-68-PSMA-11 PET/CT in 12 patients in whom conventional imaging modalities did not show lymph node involvement (40). Herleman et al. (41) demonstrated that the accuracy of Ga-68-PSMA-11 PET/CT (88%) was superior to that of CT (77%). More importantly, 40% of the lymph nodes detected via Ga-68-PSMA-11 PET/CT were reported to have short axis lengths of <5 mm (41). In a prospective study including 30 moderate/high-risk patients, the mean diameters of lymph nodes that were actually detected and those that could not be detected using Ga-68-PSMA-11 PET/CT were 4.7 mm and 2.7 mm, respectively (42). None of the currently available imaging modalities are able to accurately detect lymph nodes because of their size. However, PSMA PET is superior to conventional imaging methods in the detection of lymph nodes. With the exception of the study by Maurer et al. (40), its

specificity and sensitivity were higher than CT and MRI. PSMA PET imaging has the potential to determine N stage and thus to change initial prostate cancer stage.

PSMA Imaging for M Staging

In all previous studies, Ga-68-PSMA-11 PET/CT was used for whole-body screening. PSMA imaging detects bone and visceral metastases more accurately than conventional imaging modalities (CT and bone scintigraphy). In a retrospective study including 126 patients, Ga-68-PSMA-11 PET/CT detected osseous metastases with 99% sensitivity and 88% specificity, whereas these rates were 87% and 61% in bone scintigraphy, respectively (43).

Although PSMA PET imaging is better in M staging, it is not yet clear whether this advantage makes a positive impact in patient management.

Limitations of PSMA PET in Primary Staging

Firstly, PSMA expression in primary lesions is variable and heterogeneous, and thus, sensitivity is limited (44). Moreover, benign diseases such as prostate hyperplasia are associated with high PSMA expression and have the potential to reduce specificity (45,46). Although PSMA PET is more reliable in TNM staging compared to conventional methods, studies focusing on the clinical effect of Ga-68-PSMA-11 PET/CT at primary diagnosis are needed.

PSMA Imaging for Biochemical Recurrence

The risk of biochemical recurrence of prostate cancer is 15-20% within 5 years of first treatment and 25-30% within 10 years (47,48). Correct diagnosis and tumor location are essential because clinical management varies from active surveillance to local/systemic treatment (29). Despite important advances in imaging methods, determining the location of recurrences remains a major challenge. For patients with elevated PSA or clinical symptoms, current guidelines recommend either radionuclide bone scintigraphy, abdominopelvic CT, MP-MRI, or choline/acetate PET/CT (29). However, the recommended imaging modalities have limited detection rates (29,24). In patients with serum PSA level <7 ng/mL, the probability of having a positive bone scintigraphy is <5% (29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51). CT is positive in only 11-14% of patients with biochemical recurrence (51). Moreover, in a study of 132 patients, it was reported that in order for CT to be positive, the mean PSA value must be 27.4 ng/mL (51). In patients with high-risk prostate cancer, MRI and choline PET/CT were reported to have comparable sensitivity in the detection of bone metastasis (52). However, the rate of lymph node detection is very low (53). Depending on serum PSA levels, choline or acetate PET are reported to have a detection rate between 11-75%, and the detection range is 5-44% at PSA <1 ng/mL (54,55,56,57,58,59,60,61). Its main limitation is low sensitivity for micrometastatic disease. Therefore, choline PET/CT is now recommended for patients with biochemical recurrence and PSA >1 ng/mL (29). Ga-68-PSMA-11 PET/CT is promising for determining tumor location in patients with biochemical recurrence. There are studies reporting a high overall accuracy rate of around 54-100% for median PSA levels of 0.2-4.6 ng/mL. Moreover, the detection rate in patients with PSA level <1 ng/mL is between 44-73%. These results are also confirmed by a prospective study including 31 patients. Location of recurrence was accurately determined in 22/31 patients (71%) using Ga-68-PSMA-11 PET/CT (62). In this patient group, median PSA was reported as 2.0 ng/mL (0.1-130 ng/mL). The detection rate was found to be 47.6% in those with PSA <0.83 ng/mL (62). The rate of detection by Ga-68-PSMA-11 PET/CT increased with elevated PSA levels (63-65). However, Gleason score and neoadjuvant or adjuvant androgen therapy did not affect the detection rate (63).

Impact on Patient Management

Ga-68-PSMA-11 PET/CT has resulted in management changes in patients with early biochemical recurrence (65,66). In two different studies, management of treatment was changed in 60% and 29% of patients scheduled for radiotherapy based on the results of Ga-68-PSMA-11 PET/CT (65,66). In one of those studies, Sterzing et al. (66) reported a switch from radiotherapy to systemic treatment in only 4 of 42 patients (10%) and changes in radiation dosage and location only in the other 21 of 42 patients (50%). In the other, Van Leeuwen et al. (65) reported significant management changes in 11/20 patients (55%) who were scheduled for salvage radiotherapy. Of these patients, therapy was changed from radiotherapy to surgery in 1/20 (5%), androgen suppression treatment was added for 6/20 (30%), salvage radiotherapy was changed to extrapelvic stereotactic radiotherapy in 3/20 (15%), and stereotactic radiotherapy was added for an extrapelvic lesion in 1/20 (5%) (65). Overall, evidence to date shows that PSMA imaging has overcome the limitations of choline PET/CT and conventional imaging modalities in patients with biochemical recurrence and low PSA levels. In addition, PSMA imaging influences treatment approach in some patients with biochemical recurrence. Therefore, PSMA imaging has the potential to become routine for patients with biochemical recurrence due to its superiority over conventional imaging modalities and its role in guiding therapeutic management (66).

PSMA Therapy

Current approaches in metastatic castration-resistant prostate cancer include chemotherapy, hormone therapy, and abiraterone or enzalutamide. In addition, Radium-223 was approved for the treatment of symptomatic bone metastases. The first RLT in prostate cancer used Lu-177-J591, a monoclonal antibody with affinity for the extracellular domain of PSMA (67). Although this treatment showed promising outcomes, it was limited due to myelosuppression (67). With the development of PSMA ligands with nanoparticles, Lu-177-based radionuclide therapies are being reinvestigated in patients with metastatic prostate cancer. Some studies have yielded promising results using Lu-177-labeled PSMA ligands. The mean tumor dose is 6-12-fold higher than in the critical organs, kidneys, and salivary glands (68). Moreover, the tumor/organ ratio is higher than Lu-177-DOTATE, which is the standard RLT for NET patients (68,69). Reduction in PSA was observed in 59-89% of patients after a single dose of Lu-177-PSMA RLT using PSMA-617 or PSMA I&T. In addition, the reduction in PSA was greater than 50%

in 26.3-58.9% of the patients. Furthermore, Ga-68-PSMA PET/CT was performed on patients at 6 months after the last cycle to determine disease progression. Based on different criteria, partial treatment response was seen in 56-91%, stable disease in 0-64%, and progression in 9.1-36%. Finally, overall survival was compared in patients under Lu-177-PSMA treatment among a cohort receiving best supportive therapy. It was reported that overall survival was 29.4 weeks with Lu-177-PSMA treatment versus 19.4 weeks with best supportive care (HR: 0.44, 95% CI 0.20-0.95, P=0.031) (70). In conclusion, Lu-177-PSMA can potentially prolong life. A similar effect was shown in advanced prostate cancer using I-131-labeled MIP-1095 compound, but the data are limited (24).

Toxicity

Patients receiving Lu-177-labeled PSMA RLT experienced severe side effects. Mild and reversible side effects noted in retrospective studies included dry mouth, nausea, and fatigue (70,71,72,73,74,75,76). Heck et al. (74) reported grade 1-2 toxicity such as anemia (32%) and thrombocytopenia (25%). In another study, grade 3 anemia occurred in 2/24 patients (8.3%) (72). No marked nephrotoxicity (grade 3,4) was observed (71,72,73,74,75,76). Most patients tolerated therapy, and no acute side effects after Lu-177-PSMA injection have been reported (72,73,74,75,76). Ahmadzadehfar et al. (72) retrospectively studied adverse events in 10 patients and reported grade 3/4 hematological toxicity in 1 patient 7 weeks after RLT administration. Most patients (n=6) showed no hematological toxicity during the 8 weeks after injection (72). When compared with current chemotherapies, Lu-177-based therapies have milder side effects. In the GETUG-AFU 15 study, 38% of patients receiving chemotherapy together with androgen suppression treatment experienced severe side effects, primarily neutropenia (77).

Conclusion

Lu-177-PSMA therapy has shown promising results. It is efficient and well-tolerated, and can prolong overall survival. However, most studies have been retrospective in design. Further randomized, controlled studies are needed to demonstrate the clinical value of PSMA-targeted RLT.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: A.H., Design: D.B., A.H., Data Collection or Processing: D.B., Analysis or Interpretation: A.H., Literature Search: D.B., A.H., Writing: D.B.

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