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Introduction

- Materials and Methods/Patients and Methods
- Results
- Discussion
- Study Limitations
- Conclusion
- Acknowledgements
- References
- Tables/Figures

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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/);

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Main Text

Conclusions

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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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Original Article

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Can High Preoperative Neutrophil-lymphocyte Ratio Predict the Recurrence and Progression Risk of Non-muscle-invasive Bladder Tumors?

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Ege University Faculty of Medicine, Department of Urology, İzmir, Turkey

Abstract

Objective: Neutrophil-lymphocyte ratio (NLR) is a well-known, cost-effective biomarker of inflammatory conditions, and its protumor effect has been shown in different types of cancers. In this study, we aimed to evaluate the relationship between blood parameters, especially NLR, with the risk of progression and recurrence in non-muscle-invasive bladder tumors (NMIBT).

Materials and Methods: Seventy-six patients were included in the study. Patients were divided into low, moderate and high-risk groups according to the risk of progression and recurrence. The preoperative blood parameters of the patients were recorded from the patient files and the NLR of each patient was calculated. These parameters were compared in terms of progression and recurrence risk groups. P values less than 0.05 were accepted statistically significant.

Results: Neutrophil-lymphocyte ratio was significantly higher in the high-risk group in both the progression and recurrence risk groups than in the low and moderate risk groups ($p<0.001$). In addition, according to the post hoc results, the NLR values in the high-moderate and moderate-low risk groups showed significant differences (high-moderate and moderate-low values in terms of risk of recurrence were 4.66 vs 3.67 and 3.67 vs 2.88, respectively, $p<0.001$; high-moderate and moderate-low values in terms of risk of progression were 4.72 vs 3.68 and 3.68 vs 2.92, respectively, $p<0.001$).

Conclusion: In our study, we found that groups with high risk of recurrence and progression had higher NLR values in patients with NMIBT. NLR, which is cheap, rapid and routinely applied in preoperative evaluation, is a promising biomarker in the prognostic classification of bladder tumors. Well-designed, large-scale prospective studies with long-term follow-up are needed to determine the role of NLR in this issue.

Keywords: Neutrophil-lymphocyte ratio, non-muscle-invasive bladder tumor, progression, recurrence, prognosis

Introduction

Bladder tumor (BT) is the most common malignancy of urinary tract with the highest incidence, and it is the seventh most common malignancy in men and eleventh most common malignancy in both genders (1). Approximately 75% of BT is limited to mucosa (Ta), carcinoma *in-situ* (CIS) or submucosa (T1) at the time of diagnosis, and this rate may be higher in patients younger than 40 years (2). Treatment in non-muscle-invasive bladder tumors (NMIBT) is planned according to the prognostic characteristics of the disease. According to the scoring system and risk tables developed by the genito-urinary cancer group of European Organization for Research and Treatment of Cancer (EORTC), the number of tumors, tumor size, previous recurrence rate, T stage, presence of carcinoma *in-situ* (CIS) and tumor grade are the most important factors predicting the possibility of tumor progression and recurrence (3). Studies were carried out to determine the factors that predicted the prognosis of NMIBT and a new scoring system was established by the Spanish Urological Club for Oncological Treatment after the analysis of 1062 patients. In addition to EORTC's scoring system, the age and gender of the patient were also included in the evaluation (4). Then, in their analysis including 1812 patients who received maintenance BCG treatment, EORTC stated that previous recurrence rate and number of tumors were the most important prognostic factors for disease recurrence and that stage and grade were the most important prognostic factors for disease progression, nomograms were designed according to new risk groups (5).

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Since NMIBT is a heterogeneous disease group with different recurrence, progression and disease-related mortality rates, the planned treatment and follow-up protocol may vary according to the risk classification and preferences of the patients. Therefore, it is very important to determine the variables that can predict the risk of recurrence and progression of patients and to plan the appropriate treatment for each patient group (3).

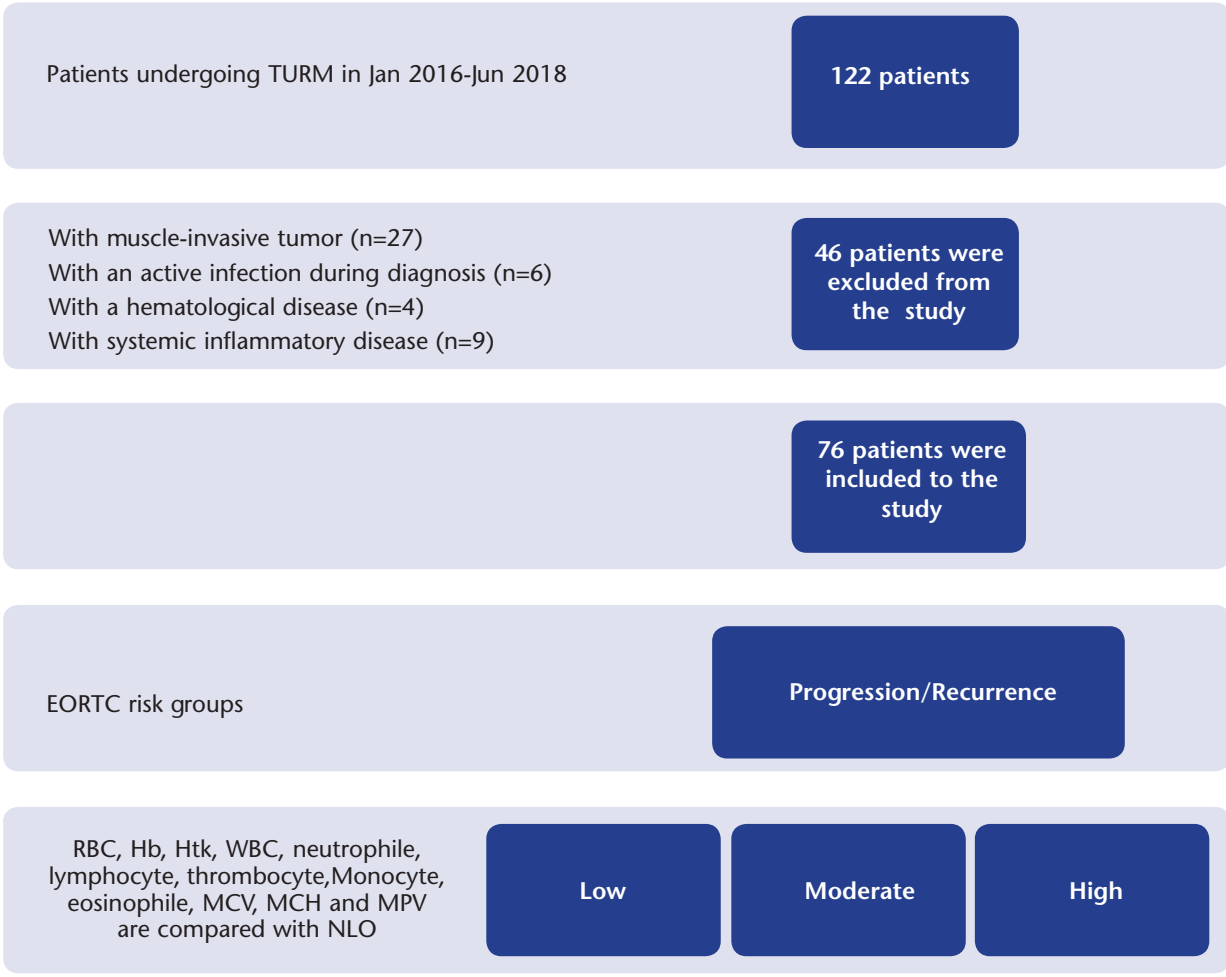
The systemic inflammatory condition triggered by cancer cells causes neutrophilia and lymphocytopenia, resulting in a tumorigenic inflammatory environment. Neutrophil-lymphocyte ratio (NLR) is a well-known and cost-effective marker of inflammatory conditions. In addition to many inflammatory conditions, high NLR has been shown to cause a worse prognosis in many different cancers such as colon, pancreas, stomach and lungs (6,7,8,9). High preoperative NLR has been shown to cause poor prognosis and pathological stage progression in bladder tumors (10). However, in most of these studies, the tumors investigated in relation to high NLR are muscle-invasive tumors and it has been shown that the increased NLR in these studies is related to the presence of muscle invasion, extravesical disease and poor cancer-specific and overall survival (10,11,12).

The aim of this study was to investigate whether high NLR is a determinant factor for progression and recurrence in patients with a diagnosis of NMIBT with transurethral resection of BT (TURB) and histopathological confirmation of non-muscle-invasion (Ta,T1).

Materials and Methods

Patient Selection and Study Design

The data of 122 patients who were operated on in our clinic between January 2016 and June 2018 with a diagnosis of BT were retrospectively analyzed. A total of 46 patients were excluded from the study, including 27 patients with confirmed histopathological diagnosis of MIBT, six patients with an active infection at the time of diagnosis, four patients with a hematological disease and nine patients with systemic inflammatory disease such as Systemic Lupus Erythematosus,



TURM: Transurethral bladder resection, EORTC: European Organisation for Research and Treatment of Cancer, RBC: Red blood cell, Hb: Hemoglobin, Htk: Hematocrit, WBC: White blood cell, MCV: Mean cell volume, MCH: Mean cell hemoglobin, MPV: Mean platelet volume, NLR: Neutrophile lymphocyte ratio

Figure 1. The flow chart of the study

Behçet or Sjögren that may affect NLR. The remaining 76 patients were included in the study. The red blood cell (RBC), hemoglobin (Hb), hematocrit (Htc), white blood cell (WBC), neutrophil, lymphocyte, platelet, monocyte, eosinophil, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean platelet volume (MPV) before TURB were recorded from patient files and NLR of each patient was calculated. Based on the histopathology of the patients after the first TURB operation, further treatments were performed as specified in the European Association of Urology (EAU) guidelines (intravesical chemotherapy or immunotherapy for NMIBT, radical cystectomy for MIBT) (13,14).

Patients were divided into low, moderate and high-risk groups according to the progression and recurrence risks after a mean follow-up period of 18.4 months. The patients' risk score for recurrence and progression was made according to the EORTC classification system based on the number of tumors, size, previous recurrence rate, T stage, concomitant CIS and tumor grade. Progression risk groups were as follows: low-risk group: a score of 0, moderate-risk group: score between 2-6 and high-risk group: score >7. Recurrence risk groups were as follows: low-risk group: a score of 0, moderate risk group: score between 1-9 and high risk group: score >10 (3,13).

The primary outcome of the study was the evaluation of the relationship between NLR and the risk of progression and recurrence, and the secondary outcome was the evaluation of the relationship between blood parameters and the risk of progression and recurrence. Before the operation, written informed consent was obtained from all patients in order to be able to use their data in scientific studies without revealing their private information. The flow chart of the study is shown in Figure 1.

Statistical Analysis

Descriptive statistics were given as mean ± standard deviation (SD). The Shapiro-Wilk test was used to check the normality of

Table 1. Demographic characteristics and tumor characteristics of patients according to recurrence and progression groups

Variables	Recurrence risk (n=76)			Progression risk (n=76)			p
Gender **Female** **Male**	Low 12 22	Moderate 10 16	High 6 10	Low 15 26	Moderate 8 14	High 5 8	0.249
Mean age ± SD (years)	52.8±3.4	56.6±4.2	61.4±5.8	53.9±4.6	58.8±5.4	63.2±5.9	***0.026***
Mean body mass index ± SD (kg/m²)	21.4±3.8	23.8±2.9	22.6±2.1	23.3±3.1	24.9±2.0	23.8±4.2	0.488
Mean tumor size ± SD (mm)	28.8±4.8	33.7±8.6	38.4±9.5	22.5±3.9	35.8±8.7	43.1±5.5	***0.019***
Mean number of tumors ± SD	0.8±0.3	1.6±1.1	2.3±1.4	1.0±0.4	2.1±0.8	3.2±0.9	***0.032***

SD: Standard Deviation Statistically significant p values were given in bold and italics

Table 2. Comparison of blood parameters of patients according to risk tables developed by European Organization for Research and Treatment of Cancer (EORTC) genito-urinary cancer group

Variables	Recurrence risk (n=76)			Progression risk (n=76)			p
	Low	**Moderate**	**High**	**Low**	**Moderate**	**High**	
The number of patients	34 (%44.7)	26 (%34.2)	16 (%21.0)	41 (%53.9)	22 (%28.9)	13 (%17.1)	-
RBC	4.86±2.2	4.46±1.9	4.18±2.3	5.02±2.4	4.59±2.1	3.99±1.8	***0.0038***
Hb	14.8±4.8	12.6±3.8	10.8±2.9	14.2±3.3	12.4±2.7	9.8±1.8	***0.011***
Htc	42.4±5.4	38.6±4.8	33.5±3.1	43.3±4.1	39.6±3.9	32.5±3.7	***0.0042***
WBC (x1000)	5.5±1.8	6.8±2.1	7.3±3.3	4.8±1.2	5.8±2.2	7.5±2.9	***0.0027***
Neutrophile (x1000)	4.2±2.3	5.6±1.9	6.6±2.1	5.4±1.8	6.5±2.6	7.9±	***0.0019***
Lymphocyte (x1000)	3.9±1.4	2.8±1.8	2.2±1.5	4.2±1.8	3.3±1.9	2.7±0.8	***0.0231***
Thrombocyte (x1000)	282.4±78.4	299±82.5	301±76.6	274.4±88.3	288.2±67.4	268.9±63.2	0.089
Monocyte (x1000)	0.78±0.4	0.74±0.5	0.76±0.6	0.72±0.5	0.71±0.6	0.69±0.4	0.368
Eosinophil (x1000)	0.26±0.3	0.28±0.5	0.29±0.4	0.27±0.4	0.31±0.5	0.26±0.3	0.454
MCV	82.5±6.8	84.6±7.2	87.5±8.4	88.5±7.5	86.4±8.2	85.3±7.8	0.238
MCH	27.4±3.3	28.3±3.8	29.5±4.1	28.2±3.1	29.2±4.1	30.8±2.9	0.062
MPV	8.56±1.2	8.71±1.1	8.82±1.3	8.64±0.9	8.91±1.2	8.74±1.4	0.071
NLR	2.88±1.8	3.67±1.7	4.66±1.9	2.92±1.1	3.68±2.1	4.72±2.7	***<0.001***

RBC: Red blood cell, Hb: Hemoglobin, Htc: Hematocrit, WBC: White blood cell, MCV: Mean cell volume, MCH: Mean cell hemoglobin, MPV: Mean platelet volume, NLR: Neutrophil-lymphocyte ratio; Values are given as mean ± SD or as number (%)
Statistically significant p values were given in bold and italics.

the distribution. The patient characteristics of the three groups were compared using Pearson's chi-square test in case of different variables. The significance of the difference between the three groups was assessed by one-way analysis of variance (ANOVA) in case of normal distribution or by Kruskal-Wallis test (non-parametric variance analysis) in case of non-normal distribution of continuous variables. Differences between two groups were determined by Bonferroni post hoc test. P values less than 0.05 were accepted for statistical significance. All statistical analyzes were performed with SPSS statistical software (Version 22.0, SPSS Inc., Chicago, IL, USA).

Results

Of the 76 patients, 48 were males and 28 were females. Twenty-two men were in the low-risk, 16 were in the moderate-risk and 10 were in the high-risk groups for recurrence. Twenty-six men were in the low-risk, 14 were in the moderate-risk and eight were in the high-risk groups for progression. Twelve women were in the low-risk, 10 were in the moderate-risk and six were in the high-risk groups for recurrence. Fifteen women were in the low-risk, eight were in moderate-risk and five were in the high-risk groups for progression. The mean age of the high-risk group was higher than the low- and medium-risk groups (52.8 vs 56.6 vs 61.4 for the recurrence, 53.9 vs 58.8 vs 63.2 for progression, p=0.026). In addition, the mean tumor size and number of tumors were higher in the high-risk group for both the risk of recurrence and progression (p=0.019 and p=0.032, respectively). Demographic characteristics and comparison of tumor characteristics of patients according to the recurrence and progression groups is summarized in Table 1.

Patients with both high risk of progression and recurrence had higher WBC values than patients with low- and moderate-risk (4.8 vs 5.8 vs 7.5 and 5.5 vs 6.8 vs 7.3, p=0.0027 for progression and recurrence, respectively). According to post hoc test results, WBC values of moderate-risk group were higher than low-risk group (p=0.0041). The RBC, hemoglobin and hematocrit values of the high-risk group were lower than the other two groups (p=0.0038, p=0.011 and p=0.0042, respectively). The same values were lower in the moderate-risk group than the low-risk group (p=0.031, p=0.029 and p<0.001, respectively). There were no significant differences in MCV, MCH and MPV values between the three groups in both risk groups.

Neutrophil-lymphocyte ratio in both risk groups was significantly higher in the high-risk group than in the low- and moderate-risk groups (p<0.001). Furthermore, according to the post hoc results, NLR values were significantly different in the high-moderate- and moderate-low-risk groups (high-moderate- and moderate-low- values for the risk of recurrence were 4.66 vs 3.67 and 3.67 vs 2.88, p<0.001, respectively, and moderate-low values were 4.72 vs 3.68 and 3.68 vs 2.92, p<0.001, respectively). A comparison of the blood parameters of patients in high-, moderate- and low-risk progression and recurrence groups is summarized in Table 2.

Discussion

Non-muscle-invasive bladder tumors, which constitute the majority of bladder tumors (75%), constitute a heterogeneous tumor group with different recurrence, progression and disease-related mortality rates (15). The treatment methods planned according to the risk groups, physician and patient preferences vary significantly. Tumors in this group have a recurrence rate of up to 70-80% and a significant rate of progression (16). Therefore, identifying patients with similar risk of recurrence and progression in these patients is very important to predict the course of the disease and oncologic outcomes, and to decide the appropriate treatment method for each patient. For this purpose, the risk classification system developed by EORTC is widely used and patients can be grouped according to the risk of recurrence and progression by one and five years according to tumor characteristics (5). Based on the risk classification of the EORTC, the International Bladder Cancer Group and the EAU Panel have developed classification systems to divide patients into low, moderate, high, and very high-risk groups to guide treatments (14,17). However, despite all efforts, the predictive level of these models with full accuracy is suboptimal to decide on optimal treatment (3). Due to the heterogeneous nature of the disease, factors are needed to predict the success of treatment and help to select the most appropriate treatment for each patient.

In recent years, the effect of inflammation on cancers has been investigated in general, and the mechanisms of carcinogenesis, progression, recurrence, metastasis and resistance have been tried to be elucidated (18). In this regard, NLR is one of the most important prognostic markers of inflammation. A recent meta-analysis of four studies with NMIBT and 14 studies with MIBT showed that preoperative NLR was associated with recurrence-free (HR=1.58) and progression-free survival (HR=1.33) (19). In the literature, there are few studies on the prognostic significance of NLR in MIBT and there is limited number of studies evaluating the relationship with NMIBT (10,20,21).

The meta-analysis of six studies with a total of 2.298 patients demonstrated that NLR level in patients who underwent TURB because of NMIBT was a risk factor for increased disease recurrence and progression. In addition, NLR has been reported to be an independent predictor of disease recurrence and progression in NMIBT patients receiving BCG treatment (22). Identifying patients at risk for recurrence and progression in NMIBT patients contributes to the selection of the most appropriate candidates for treatment method such as intravesical BCG therapy or a more invasive radical cystectomy and optimization of the follow-up protocol of these patients. These results should be supported by well-designed, prospective randomized studies. There is no generally accepted NLR threshold-value in studies. Most researchers used the threshold-value determined by the highest specificity and sensitivity determined by the statistical method used, while others used threshold-values previously defined in the literature. In our study, NLR values differed significantly regarding prognostic groups, and unlike previous studies, a specific threshold-value was not used for NLR. In general, the threshold-value accepted or determined was over two.

BT is known to be an immunogenic malignancy and intravesical

BCG is widely used for its treatment. BCG treatment has been shown to reduce the risk of recurrence and progression, particularly in the high-risk NMIBT group. The immunological system has an antagonistic effect in the pathogenesis of BT, while the acquired immune system has an anti-tumor effect, and the hereditary immune system has a pro-tumor effect (23). High NLR is believed to contribute to carcinogenesis by promoting tumor aggression by increasing the number of neutrophils by interacting with other groups of cells, producing cytokines and effector molecules. Neutrophils are capable of rapidly generating host responses by chemokines, pathogenic signals and lipid mediators. Major tumorigenic effects include cell invasion, cancer cell proliferation, lymphangiogenesis, and re-generation of the matrix outside the cell. On the other hand, anti-or pro-tumor effects of neutrophils, which are highly mobile cells, may also vary according to their microenvironment (24).

Mano et al. (25) evaluated the prognostic significance of NLR in 122 NMIBT patients who underwent TURB in their study and found that NLR over 2.41 was associated with disease progression and above 2.43 was associated with disease recurrence. In this study, similar to our study, the patients were classified according to the risk groups of EORTC and it was found that the number of patients with high NLR was higher in the high-risk group both in the progression and recurrence groups. The relationship between NLR and subgroups of T stage at the time of diagnosis was also evaluated and it was shown that NLR of patients with lamina propria invasive histopathology (T1) was higher than the non-invasive (Ta) group and that lymphocyte count was lower (26). In a retrospective study of the data of 1,551 patients who underwent TURB with a diagnosis of NMIBT in a single center from Korea, it was shown in multivariate analysis that high NLR is an independent predictor for both general and cancer-specific survival and may be an important predictor of oncologic outcomes, especially mortality (27).

In a study in 222 patients with MIBT and NMIBT, Celik et al. (28) concluded that NLR is a predictive biomarker and found significantly higher NLR in the MIBT group than in the NMIBT group. In addition, RBC, Hb and Htc values were lower in the MIBT group. In our study, although all patients had NMIBT, these parameters were similarly significantly lower in the high-risk group. The fact that patients in the high-risk group complain more frequently of hematuria because they have more and larger tumors is the likely cause of this difference. These patients require more frequent hospitalization due to massive hematuria and are more frequently confronted with erythrocyte transfusion.

Recently, the effect of tumor-infiltrating immune cells on BT prognosis has been the subject of research. It has been demonstrated that tumor-infiltrating neutrophils and NLR are negative predictors and tumor-infiltrating lymphocytes are positive predictors (29). Ethnicity has also been shown to affect NLR. It was shown that NLR was associated with advanced tumor stage in 297 patients with NLR evaluation during TURB, but that European patients had higher NLR than African patients, and it was underlined that ethnicity is a factor to consider when interpreting NLR (30).

Study Limitations

There are some limitations of our study. The retrospective nature of our study and the relatively low number of patients in the groups are the main ones. The follow-up period of the patients was not long enough, but there was sufficient time for risk classification. In addition, because the NLR is a marker differentiating according to ethnicity, the results of this study with our own population should be carefully adapted to other ethnic groups. On the other hand, we believe that our study is valuable because it is one of the rare studies in the literature in which NLR classification of NMIBT patients according to EORTC risk groups is made.

Conclusion

The discovery of new predictive factors that accurately predict the prognosis of NMIBT, a highly heterogeneous group of patients, seems to be an important necessity in the field of urological oncology. Neutrophil-lymphocyte ratio is a promising, cost-effective and rapid biomarker in this regard, which is routinely applied in the pre-operative evaluation. In our study, we found that the groups with higher risk of recurrence and progression had higher NLR values in NMIBT patients. Neutrophil-lymphocyte ratio can be used in prognostic classification of these patients, in determining the treatment method and in estimating the state of muscle-invasion. In order to determine the potential role of NLR in the clinical decision stage, well-designed, large-scale, prospective studies with long-term follow-up are needed.

Ethics

Ethics Committee Approval: Because of the study was designed as a retrospective study, ethics committee approval was not obtained.

Informed Consent: Written informed consent was taken from all patients in order to be able to use their data in scientific studies without revealing their private information.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.K., A.Ş., Concept: F.K., A.Ş., Design: F.K., A.Ş., Data Collection or Processing: F.K., Analysis or Interpretation: F.K., A.Ş., Literature Search: F.K., Writing: F.K.

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Original Article

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Risk Factors Affecting Complications Due to Prostate Biopsy

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Abstract

Objective: Currently, transrectal ultrasound (TRUS) guided prostate biopsy is the standard method used for prostate cancer detection. In the last decade, hospitalization due to complications has increased, especially due to infectious causes. Therefore, it is important to determine the risk factors affecting the complications of prostate biopsy.

Materials and Methods: One hundred and sixty-four patients who underwent TRUS-guided prostate biopsy due to prostate cancer suspicion were included in our study. Patients' ages, total and free prostate specific antigen (PSA) levels, prostate volumes, digital rectal examination findings, level of education, pathology results and pain related to the procedure were recorded. A 10-cm long visual analogue scale (VAS) was used to assess the pain of the patients. Complications related to the procedure were questioned firstly on the same day and secondly during the visit of the patient for pathology. As a result of these evaluations, complications divided into three groups as none, minor (no intervention) and major (medically or surgically treated).

Results: In our study, minor complications included rectal bleeding in 42 patients and hematuria lasting longer than 48 hours in 11 patients. Major complications were fever of >38 °C in two patients and epididymitis in one patient. There was no statistically significant effect of age, total and free PSA, prostate volume, level of education, digital rectal examination findings and pathology results on complications. There was no statistically significant relationship between VAS pain score and rectal bleeding, hematuria, epididymitis. On the other hand, a statistically significant relationship was found between VAS pain score and fever.

Conclusion: In the limited number of studies on the determination of risk factors for complications associated with prostate biopsy, the level of education, digital rectal examination findings, and pain due to the procedure were evaluated. In the light of our results, we believe that patients with high pain scores may be at risk for complications, especially for fever.

Keywords: Prostate biopsy, complications, risk factors

Introduction

Prostate cancer is the second most common cancer among men worldwide (1). Currently, transrectal ultrasound (TRUS) guided prostate biopsy is the standard method used for prostate cancer detection. In recent years, there has been an increase in the number of prostate biopsies and consequent complications due to prostate biopsy in younger patients, widespread use of prostate specific antigen (PSA) worldwide, and prolonged human life. Infection rates increase with recurrent biopsies due to active follow-up (2). In the last decade, hospitalization due to complications has increased, especially due to infectious causes (3). Therefore, it is important to determine the risk factors affecting the complications of prostate biopsy. In our study, the effects of age, total and free PSA, prostate volume, level of education, pain related to the procedure, digital rectal examination findings and pathology results on complications were evaluated.

Materials and Methods

Our study was prospectively designed and 164 patients who applied to our clinic between January 2012 and May 2012 and underwent prostate needle biopsy with TRUS for suspected prostate cancer were included in our study.

Our study was approved by the ethics committee of our hospital (no: 2012/9/3) and all patients included in the study were informed about TRUS guided prostate biopsy and complications. Written informed consent was obtained from the patients.

In our study, having abnormal rectal examination and/or serum PSA levels above 2.5 ng/mL formed our indication for prostate

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biopsy. Exclusion criteria were as follows: a) patients with painful conditions of the prostate, rectum or anus, such as acute prostatitis, prostadinia, hemorrhoid, anal fissure or stricture; b) patients having neurological disorders, such as lower limb paraplegia, with decreased or diminished pain sensation; c) patients with bleeding diathesis; d) patients using analgesics, anxiolytic or narcotic drugs; and e) previous TRUS-guided prostate biopsy. Anticoagulant, antiaggregant and thrombolytic drugs were discontinued at least one week prior to prostate biopsy.

The patients' ages, total and free PSA levels, prostate volumes, digital rectal examination findings, levels of education, pathology results and pain related to the process were recorded and the effects of these data on the complications were evaluated statistically for each complication type separately and for all the complications.

Digital rectal examination findings of the patients were evaluated as benign or suspicious. The patients with the findings of stiffness, nodule, irregularity, loss of sulcus etc. in digital rectal examination were evaluated in the suspicious group. Patients were divided into two groups in terms level of education as below eight years of compulsory education (primary education or lower) and above eight years of compulsory education (higher than primary education). Pathology results of the patients were recorded as benign or malignant.

A 10-cm long visual analogue scale (VAS) was used to evaluate the pain of the patients. On this scale, the starting point zero (0) describes no pain and the end point ten (10) describes the most severe pain experienced. Following the explanation of the VAS by the physician, the patients were asked to give a point on the scale for the pain they felt. In order to prevent incorrect pain scoring, the biopsy shot sound was listened before the procedure and the patients were told not to take this sound into consideration. All the informing about VAS and biopsy applications were performed by the same physician. The data obtained by measuring the distance of the marks on the scale to the zero starting point were measured in millimeters as pain scores. Pain score measurements were made immediately after the biopsy procedure was completed and the rectal probe was removed.

The patients were positioned in the left lateral decubitus position and the hips and knees were flexed. The "LOGIQ 100 PRO Series" ultrasound device equipped with a 6.5 MHz rectal probe with a maximum diameter of 23 mm was used for TRUS imaging. Once the probe was placed rectally, the prostate was visualized in the sagittal and transverse plane and the prostate volume was automatically calculated with the ellipsoid formula on the ultrasound instrument. The anesthetic agent for periprostatic nerve blockade was injected with a 30 cm 18 gauge (G) spinal needle in the sagittal plane in 5 cc doses separately into the region of both neurovascular bundles between the prostate base and the seminal vesicle after checking to prevent intravascular injection. After periprostatic nerve blockade, an 18 gauge 30 mm automatic biopsy gun was used to obtain specimens from 12 cores from the posterolateral region of the peripheral zone in accordance with the European Association of Urology (EAU) guideline. Since all patients in our study were biopsied for the first time, no transitional zone (TZ) sampling was performed. In all patients, 12 core biopsy specimens were taken at the same anatomical order.

All patients took ciprofloxacin (500 mg) orally twice a day for one day before and four days after biopsy. A fleet enema was performed rectally to each patient in the morning before biopsy.

After the procedure, all patients were kept for at least one hour and complications were recorded. Patients with no problem were discharged. The second evaluation of the patients for complications was made during their visit for pathology results. As a result of these evaluations, complications were divided into three as no complication, minor (no intervention) and major (medically or surgically treated) complications. Patients were advised to admit the hospital in cases of high fever (≥38°C), dysuria, hematuria or rectal bleeding.

Statistical Analysis

Independent sample t-test was used for the quantitative data having normal distribution. Kruskal-Wallis test was used for quantitative data that did not have normal distribution. Pairwise comparisons were made with Mann-Whitney U test. Qualitative data with independent variables were evaluated with chi-square test and Fischer's exact test. $P<0.05$ was considered statistically significant.

Results

The mean age of the patients was 66.1 years. The median value for total PSA was 8.8, the median value for free PSA was 1.5, the median value for prostate volume was 64 and the median value for VAS pain score was 10. While the number of patients with lower education level was 133, the number of patients with higher education level was 31. The number of patients with benign digital rectal examination findings was 89 and the number of patients with suspicion was 75. The numbers of patients with benign and malignant pathology were 130 and 34, respectively. The standard deviation, minimum, maximum and percentage ratios for this data are shown collectively in Table 1.

In our study, minor complications included rectal bleeding in 42 patients and hematuria lasting longer than 48 hours in 11 patients. Major complications were high fever in two patients and epididymitis in one patient. All of the patients had stopped rectal bleeding at the first hour. Rectal hemorrhage and hematuria were evaluated as Clavien grade 1 complications, and high fever and orchitis as Clavien grade 2 complications. Hematospermia, vasovagal episode, urinary retention and bacterial sepsis, which are other complications due to prostate biopsy, were not seen in our study. The numerical and percentage distribution of the complications is shown in Table 2.

There was no statistically significant relationship between age, total-free PSA, prostate volume, level of education, digital rectal examination findings and pathology, with rectal bleeding, hematuria, fever and epididymitis. When all the complications were evaluated together, no statistically significant results were found for these parameters. There was no statistically significant relationship between VAS pain score and rectal bleeding, hematuria, epididymitis and all complications; however, a

statistically significant relationship was found for high fever. P values for these results are shown in Table 3. The relationship between VAS pain score and high fever was evaluated by ROC analysis. Accordingly, a cut-off value of >46.5 for VAS pain score was found to be a value for possible complications (AUC=0.935).

Discussion

In their study evaluating the complications related to prostate biopsy, Rietbergen et al. (4) reported that rectal bleeding with increasing age tended to increase slightly but this was not significant. In our study, no significant relationship was found between age and both rectal bleeding and other complications. This situation is similar to many studies in the literature (3, 5,6,7,8,9). Again, in three studies that age was not a risk factor for complications, a negative correlation was found between age and hematospermia (4,10,11). This finding was explained by the decrease in the sexual activity of the patients with increasing age. In our study, hematospermia was not seen, however, hematospermia was found to be 37.4% according to the guidelines of the EAU. We think that this difference in our study is due to the fact that the number of patients having sexual intercourse may be low in this period which can be considered as early after the biopsy since the patients' inquiries about the complications were made in the visits they came to show the pathology results after about two weeks.

There was no significant relationship between prostate volume and any complications seen in our study. There are studies reporting similar results in the literature (5,7,8,9,12). However, Loeb et al. (13) found a significant relationship between prostate volume and fever. In this study, the patients between 1993 and 2011 were examined and trimethoprim-sulfamethoxazole was used for prophylaxis until 2008 and ciprofloxacin was used after this date. Ciprofloxacin was continued for five days only in high-risk patients. In the same study, it was reported that trimethoprim-sulfamethoxazole resistance was around 80% in patients who were hospitalized and from whom urine/blood cultures were obtained. In our study, ciprofloxacin was administered to all patients for a total of five days. We think that the difference between the two studies is related to different protocols applied in prophylaxis. Shigemura et al. (14) found a significant relationship between prostate volume and infectious complications. In this study, TZ sampling was performed in 51 patients (42.5%) and bowel cleansing was

Table 1. Mean, standard deviation, median, minimum, maximum, numerical and percentage data calculated for variables

Age, mean (± SD)		66.1 (±8.66)
Total PSA, median (minimum-maximum)		8.8 (1-314)
Free PSA, median (minimum-maximum)		1.5 (0-66)
Prostate volume, median (minimum-maximum)		64 (13-256)
VAS pain score, median (minimum-maximum)		10 (2-97)
Level of education	Primary education or lower, n (%)	133 (81.1%)
	Higher than primary education, n (%)	31 (18.9%)
	Total, n (%)	164 (100%)
Digital rectal examination	Benign, n (%)	89 (54.3%)
	Suspicious, n (%)	75 (45.7%)
	Total, n (%)	164 (100%)
Pathology report	Benign, n (%)	130 (79.3%)
	Malignant, n (%)	34 (20.7%)
	Total, n (%)	164 (100%)

PSA: Prostate specific antigen, VAS: Visual analogue scale, SD: Standard deviation
n: Number of the patients

Table 2. Numerical and percentage distribution of complications

Minor complications Clavien grade 1						**Major complications Clavien grade 2**					
Rectal bleeding, n (%)			**Hematuria, n (%)**			**Fever, n (%)**			**Epididymitis, n (%)**		
Yes	No	Total	Yes	No	Total	Yes	No	Total	Yes	No	Total
42 (25.6%)	122 (74.4%)	164 (100%)	11 (6.7%)	153 (93.3%)	164 (100%)	2 (1.2%)	162 (98.8%)	164 (100%)	1 (0.6%)	163 (99.4%)	164 (100%)

n: Number of the patients

Table 3. P values calculated for the effects of variables on complications

	p				
	Rectal bleeding	**Hematuria**	**Fever**	**Epididymitis**	**All complications**
Age	0.350	0.731	0.892	0.321	0.602
Total PSA	0.286	0.229	0.858	0.874	0.518
Free PSA	0.434	0.173	0.946	0.899	0.557
Prostate volume	0.605	0.308	0.495	0.899	0.752
Level of education	0.654	0.209	1	1	0.414
Digital rectal examination findings	0.630	0.643	0.498	1	0.540
Pathology report	0.177	1	1	1	0.606
VAS pain score	0.356	0.783	**0.037**	0.332	0.190

PSA: Prostate specific antigen, VAS: Visual analogue scale

not performed with rectal enema. In our study, since we included patients who underwent biopsy for the first time, TZ was not sampled in accordance with the guidelines of the EAU, and all patients underwent bowel cleansing with rectal enema on the morning of biopsy. In our study, infectious complications such as acute prostatitis and sepsis have not been observed and there are studies in the literature indicating that there is no relationship between prostate volume and these complications (15,16,17). Although we did not observe complications such as urinary retention and syncope in our study, there are studies in the literature that correlate prostatic volume with these complications (6,10,11,18). Some studies have shown a significant relationship between prostate volume and hematuria (10,11,19). In these studies, Raaijmakers et al. (10) performed prostate biopsy without any anesthesia. We think that application of this procedure without anesthesia, in which patients feel a great amount of pain, affects the hematuria rates. Because Obek et al. (20) reported that periprostatic nerve blockage reduces rectal bleeding. They explained this situation in a similar way to Rodríguez and Terris et al. (12), who stated that the pain felt by the patients was proportional to the rectal bleeding. In another study, Zaytoun et al. (11) sampled a mean of 15.2 cores and prostate biopsy was performed even though the patients continued to receive anticoagulant and antiplatelet drugs. In this study, we believe that the mean number of cores and the use of drugs that may cause bleeding diathesis affected the relationship between prostate volume and hematuria. Chiang et al. (6) and Namekawa et al. (18) reported no significant relationship between hematuria and prostate volume, similar to our study. In the literature, the rate of rectal bleeding seen after prostate biopsy ranges between 1.3-13% and the rate of hematuria ranges between 10-84% (21). In our study, these rates were 25.6% and 6.7%, respectively.

In our study, PSA levels did not significantly affect complications. As far as we know, other studies in the literature also report similar results (5,15,18). Simşir et al. (15) reported no significant relationship between sepsis and PSA levels, and Namekawa et al. (18) reported no significant relationship between both urinary retention and hematuria and PSA levels.

Almost all studies have shown that there is no significant relationship between pathology results and complications (7,8,9,16,22,23,24,25). To the best of our knowledge, only Rietbergen et al. (4) reported that hematuria and hematospermia rates were significantly lower in patients diagnosed with prostate cancer. They interpreted this result as the increasing threshold for reporting these complications in patients receiving bad news. Supporting this situation, Rodríguez and Terris et al. (12) they stated that the pathology result for the complications was not a risk factor in their evaluation before the pathology result was reported. The results of our study are in parallel with the vast majority of the literature.

To the best of our knowledge, the relationship between digital rectal examination (DRE) and complications was only examined by Namekawa et al. (18). In this study, there was no relationship between hematuria and DRE, and there was a significant relationship between urinary retention and DRE findings. In our study, urinary retention was not observed and there was no significant relationship between DRE and any complication including hematuria.

As a result of our study, no significant relationship was found between levels of education and complications. As far as we know, this assessment has not been done in any previous study.

There are studies in the literature showing the relationship between pain and complications (25,26,27). In the study of Celebi et al. (27), it was stated that the mean pain scores were higher in the patients with complications. Similar to this study, Djavan et al. (25) stated that patients with rectal bleeding were more likely to have pain than the patients with other complications. However, in one of these studies, no anesthesia was reported, while in the other, only rectal lidocaine gel was applied. We think that there is a relationship between rectal bleeding and pain due to these anesthesia methods, which may be considered as insufficient with the current guidelines. Because, similar to our study, Hossack and Woo et al. (28), which performed periprostatic nerve blockage, did not find any relationship between bleeding and pain scores. There was no relationship between pain and infection in this study; however, there was a significant relationship between pain scores and fever in our study. The cut-off value for pain score was 46.5. Accordingly, the likelihood of fever is significantly increased in patients with pain scores above this value. However, the fact that this value was determined as a result of the evaluation of two patients with fever suggests that new studies are needed. In EAU guidelines, epididymitis was reported as 0.8% and fever as 0.7%. In our study, these rates were 1.2% and 0.6%, respectively, and they were consistent with the guideline.

Study Limitations

The limitations of our study were as follows: a) the cut-off value for high fever regarding pain score was calculated only in two patients, b) lack of assessment of comorbidities that may affect the complications of patients, c) lack of multivariant analysis because there was a relationship between pain scores and complications only, and d) low number of cases.

Conclusion

As prostate biopsies are frequently applied in urology practice, it is important to determine the risk factors for prostate biopsy-related complications. In this study, we believe that the evaluation of the level of education, digital rectal examination findings and pain related to the treatment in this study contribute to the literature, as these were previously evaluated in a limited number of studies. Again in the light of our evaluations, we believe that patients with high pain scores may form a risky group in terms of complications, especially fever. However, in order to increase the scientific value of these results, we think that new studies with larger patient population are needed.

Ethics

Ethics Committee Approval: This study was approved by the ethics committee of University of Health Sciences, Bursa Higher Specialization Training and Research Hospital (no: 2012/9/3) and all patients included in the study were informed about

TRUS guided prostate biopsy and complications.

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A., V.Ç., M.Ş., Concept: S.A., S.Ö., V.Ç., Design: S.A., S.Ö., M.K., Data Collection or Processing: S.A., V.Ç., E.Ö., Analysis or Interpretation: S.A., E.Ö., S.Ö., Literature Search: S.A., M.Ş., M.K., Writing: S.A., S.Ö.

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Original Article

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Transurethral Resection of the Bladder Tumor Success Rates of Surgeons and Possible Causes of Differences Between Locals and Refugees

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Abstract

Objective: In our study, we examined the success rates of our surgeons and the criteria that define the success, and tried to reveal the possible causes of the differences in the success rates of transurethral resection of the bladder tumor (TUR-BT) in locals and refugees.

Materials and Methods: Between 2014 and 2018, 246 patients who underwent TUR-BT for the first time due to bladder tumor were evaluated retrospectively. Patients with urothelial carcinoma were included in the study. The patients were classified as muscle-invasive or non-muscle-invasive bladder cancer according to the stage, and as detrusor muscle positive and negative according to the pathology results. The patients were divided into two groups as locals and refugees. The surgeons were coded with numbers.

Results: The number of patients with positive detrusor muscle was 85 (52.1%) in locals and 55 (66%) in refugees. When all the cases were taken into consideration, it was found that the surgeons had significantly higher rates of detrusor muscle sampling in refugees compared to locals (p=0.006).

Conclusion: Our study suggests that the quality of the bladder tumor resection can be measured by the success or failure in sampling of the detrusor muscle in the first TUR-BT where the tumor is completely resected. The success rates of surgeons were higher in refugees. Despite the fact that they are the same group of patients, surgical treatment of the refugees without surgical stress seems to be the possible reason for being more successful.

Keywords: Bladder tumor, defensive surgery, detrusor muscle, TUR-BT

Introduction

Bladder tumor (BT) is the most common tumor of urothelial cancer. Approximately 75% of BTs are limited to bladder mucosa or submucosa at the time of initial diagnosis. Tumors in this category are called non-muscle-invasive bladder cancer (NMIBC). Tumors with detrusor muscle (DM) invasion are defined as muscle-invasive bladder cancer (MIBC) (1,2).

The standard treatment of NMIBC is intravesical instillation therapy according to the presence of risk factors following complete transurethral resection of the BT (TUR-BT). If the pathology after TUR-BT is reported as MIBC, the treatment approach changes completely and becomes radicalized (cystectomy, radiotherapy). The worse prognosis and the different treatment modality in MIBC indicate the importance of TUR-BT operation (3,4).

In addition to the standard risk factors for recurrence and progression of NMIBC, the quality of TUR-BT and surgical style are significantly effective. The most important factors in evaluating the quality of TUR-BT have been defined as surgical approach, experience and obtaining adequate pathological material (DM sampling) (3,5).

The aim of TUR-BT is to make the histological diagnosis of the BT, to determine the tumor stage-grade, to determine all the prognostic factors such as the number and size of the tumor, muscle invasion (MI), and ultimately to remove the NMIBC completely (6,7).

If the initial resection is insufficient or there is no muscle tissue in the sample, re-TUR-BT should be performed within 2-6 weeks. In order to avoid applying re-TUR-BT, it is necessary to make a complete resection and a good tumor base sampling in the first resection. The leading causes of inadequate resection and low staging are tumor size, multifocality, inadequate equipment-surgical experience, surgeons avoiding the complications and being afraid of the complication management. Complications caused by TUR-BT are lower urinary tract symptoms, bleeding,

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bladder perforation, urethral stricture and ureteral orifice obstruction. (7,8).

We have approximately 400,000 refugees in the province of Hatay. In addition to the refugees in our province, we also provide health services in Hatay State Hospital for patients referred from the field hospitals in Syria. The examination, treatment and surgeries of the refugees and locals are provided with equal opportunities without any discrimination. Although there are a large number of refugees in our country, the number of publications related to the surgeries in refugees is extremely limited. This research is the first surgical study comparing refugees and locals in Turkey.

In this study, we aimed to evaluate the factors determining the DM sampling by TUR-BT and therefore the success of the surgery on the individual surgeon level and patient groups in the treatment of patients with BT.

Materials and Methods

Patients who underwent TUR-BT due to BT in our clinic between 2014 and 2018 were evaluated retrospectively. In total, 246 patients underwent TUR-BT for the first time. Patients with urothelial carcinoma were included in the study. Patients were classified according to pathology results (positive or negative DM) and stage (MIBC or NMIBC). The patient groups were divided into two groups: local patients (LP) and refugee patients (RP). Tumor size was grouped as <10 mm, between 10-30 mm and >30 mm. Operative time was grouped as <30 min, between 30-60 min and >60 min. The surgeries performed by six different surgeons with similar experience in endoscopic bladder surgery were evaluated according to DM positivity in TUR-BT pathology samples, LP and RP, surgeon, specimen size (obtained from pathology reports) and operative time. In the imaging (CT,US) reports of 246 patients included in the study, tumor tissue was reported to be localized to the bladder. Surgeons were coded by numbering. Surgeons were evaluated statistically in terms of parameters such as detrusor muscle sampling, operative time, tumor size, and LP/RP. Surgeons used the same endovision system with the same resection elements (Karl Storz resectoscope and 30 degrees optics) during endoscopic BT resection. TUR-BT was performed under spinal anesthesia with premedication almost in all patients. General anesthesia was applied only to patients who were not suitable for spinal block.

The complications of TUR-BT reported by the surgeons were graded according to the Clavien classification, and LP/RP, tumor size and operative time were compared.

Statistical Analysis

Statistical analysis of the study was performed by R 3.4.3 program. Descriptive statistics for continuous variables in the study were expressed as mean, standard deviation, median, minimum and maximum values; and categorical variables were expressed as frequency and percentage. Yates chi-square and Pearson chi-square tests were used to compare categorical variables among groups. In all statistical analyzes, results with a p value less than 0.05 were considered statistically significant.

Results

In our clinic, 246 (233 male, 13 female) patients underwent TUR-BT between 2014-2018. The mean age of the LP was 62 years and RP was 66 years (range, 32-96). No statistically significant difference was found between LP and RP in terms of age (p=0.217) (Table 1). There was no statistically significant difference between LP and RP in terms of MI (p=1.000). There was no statistically significant difference between LP and RP in terms of tumor size (p=0.335). There was no statistically significant difference between LP and RP in terms of operative time (p=0.682).

There was no statistically significant difference between the surgeons in terms of TUR-BT numbers and the number of LP/RP operated (p=0.421). The number of patients with positive DM in TUR-BT specimen was 85 (52.1%) in LP and 55 (66%) in RP

Table 1. TUR-BT patient data

		n	%
Gender	Male	233	94.7
	Female	13	5.3
Nationality	Locals	163	66.3
	Refugees	83	33.7
Age		**Mean**	**Minimum-Maximum**
	Locals	66	32-96
	Refugees	62	33-87

TUR-BT: Transurethnal resection of blodder turnor

Table 2. TUR-BT surgery data and pathology results

		Locals	Refugees	p
Pathological stage	Non-muscle invasion	138 (84.7%)	71 (85.5%)	1.000
	Muscle invasion	25 (15.3%)	12 (14.5%)	
Tumor size	<10 mm	29 (17.8%)	10 (12.0%)	0.335
	10-30 mm	61 (37.4%)	38 (45.8%)	
	>30 mm	73 (44.8%)	35 (42.2%)	
Pathology specimens	Detrusor muscle +	85 (52.1%)	55 (66.3%)	0.006
	Detrusor muscle -	78 (47.9%)	28 (33.7%)	
Operative time	<30 minute	24 (14.7%)	9 (10.8%)	0.682
	30-60 minute	88 (54.0%)	48 (57.8%)	
	>60 minute	51 (31.3%)	26 (31.3%)	
Surgeon	Surgeon 1	23 (14.1%)	11 (13.3%)	0.421
	Surgeon 2	36 (22.1%)	15 (18.1%)	
	Surgeon 3	23 (14.1%)	18 (21.7%)	
	Surgeon 4	31 (19.0%)	11 (13.3%)	
	Surgeon 5	20 (12.3%)	15 (18.1%)	
	Surgeon 6	30 (18.4%)	13 (15.7%)	

TUR–BT: Transurethral resection of the bladder tumor

(Table 2). When all cases were taken into consideration, it was found that the rates of TUR-BT DM sampling of surgeons were significantly higher in favor of RP compared to LP (p=0.006). There was no statistically significant difference between the surgeons in terms of DM positivity within the LP and RP (p=0.194 and p=0.756, respectively).

The complications of surgeons were evaluated according to Clavien classification, and significant data could not be obtained, as high-grade complication was rare. Only surgeon 1,2 and 5 reported an extraperitoneal bladder perforation due to obturator nerve reflex.

Discussion

Transurethral resection is defined as the gold standard for BT treatment. In the first TUR-BT, all visible tumors should be removed and the presence, depth and type of tumor invasion should be determined. Quality of TUR-BT affects the diagnosis, treatment and even the prognosis of BT. Defining the TUR-BT quality criteria is extremely important in determining the treatment plan. In clinical studies, there is a general acceptance that TUR-BT is successful if the staging of the disease is evaluated correctly, namely lack of misstaging or overlooked NMIBC lesions, and if there is no complication (9,10). In our study, we compared the TUR-BT success rates by evaluating DM sampling rates according to surgeons and patient groups. We have tried to reveal the possible relationship between the different success rates of TUR-BT and defensive surgical attitude according to surgeons and patient groups.

In many clinical studies, it has been shown that the success of TUR-BT is parallel to the presence of DM in the specimen. Detection of DM in specimen is relatively easy and allows us to evaluate the quality of resection much earlier than findings in the first control cystoscopy. Tumor base sampling was standardized in TUR-BT to obtain DM. The absence of DM in the first TUR-BT shows a poor quality resection and re-TUR-BT should be performed within 2-6 weeks (11).

In our study, although there was a significant difference in the presence of DM in specimen between LP and RP, we did not find a relationship in terms of age, gender and operative time. In this case, it is understood that DM sampling varies depending on the surgical attitude more than the individual characteristics of the patients. The surgeons do not appear to act with the necessary surgical self-esteem during the surgery in LP group. In studies, it was shown that 30-50% of the pathology samples do not contain DM (3,12).

Residual tumor and high early recurrence rates following low-quality TUR-BT can be explained by the individual effect of surgeons as well as the variability in TUR-BT quality. In our study, we evaluated the DM sampling rate of six different surgeons by TUR-MT and the differences between LP and RP. There was no significant difference between surgeons in the rate of DM sampling in RP (55-81%). Similarly, there was no significant difference in LP (34-66%). When all cases were taken into consideration, it was found that surgeons had significantly higher rates of DM sampling in RP compared to LP. The fact that there was no significant difference between LP and RP in terms of muscle invasion and tumor size indicates that the tumor structure of the two groups is similar. It is possible to interpret the higher rates of DM sampling in RP by lack of surgical stress and defensive surgical attitude directed by the anxiety of complications.

In a study of 209 NMIBC patients, Del Zingaro et al. (13) reported that the high surgical volume was predictive for recurrence and progression. However, in our study, DM positivity was not correlated with tumor size among surgeons or groups (LP,RP). Similar results have been reported showing that the surgical volume did not have a significant effect on recurrence or progression rates, as in our study (14). Brausi et al. (15) reported that there was a difference between the clinics in the success of TUR-BT and that this was due to surgical experience rather than tumor characteristics. In a study of residual tumors, Herr (10) detected residual tumor in 83% of patients with negative DM in the first TUR and 74% of patients with positive DM. While similar success rates are expected among the groups of patients with the same tumor characteristics and operated by surgeons with equivalent surgical experience, the most probable cause of statistically significant differences is the complication avoidance reflex. Contrary to this attitude of surgeons, no serious complications were observed in both groups. This shows that defensive surgical attitude does not have a significant data and literature support. It has been determined that experienced surgeons sample more positive DM and that lack of DM positivity predicts the earlier recurrence risk independently (17). Dalbagni et al. (16) reiterated that the quality of TUR-BT can be measured by determining the completeness of the resection, the ability to obtain resection specimen and the recurrence at the resection site (17).

In our study, we aimed to reveal the importance of DM sampling in TUR-BT and the factors affecting it, such as tumor size and surgical attitude. In the study of Mariappan et al. (11), 67% of 365 TUR-BT patients had DM positivity. In multivariate analysis, large tumors, high-grade tumors, and surgical experience were found to be independently associated with DM positivity in resected samples (11).

Complications after TUR of the bladder have been reported in about 5-6% of patients. The frequency of complications is higher in large tumors, multifocal tumors and tumors in the bladder dome and is also dependent on the surgeon's experience. The most common complication is bleeding and occurs in 2.5% of cases. A more serious complication is bladder perforation and has been reported in 1-3% of patients. Perforation may occur as a result of obturator nerve stimulation with muscle contraction and rapid movement of the lower extremity (18,19).

In our study, TUR-BT complications of the surgeons were evaluated according to Clavien complication classification, and high-grade complications (Clavien stage 3-4) were rare, so significant data could not be obtained. Only surgeon 1,2 and 5 reported an extraperitoneal bladder perforation due to obturator nerve reflex. These results are consistent with the literature.

Study Limitations

The main limitations of the study are retrospective nature of the study, and lack of follow-up of residual tumor, recurrence and prognosis.

Conclusion

The absence or presence of DM in the first complete TUR-BT sample can be considered as an indicator of resection quality by independently predicting the presence of residual tumor related to the surgeon's experience. The success rates of surgeons in TUR-BT between LP and RP are high in favor of RP and this seems to be most likely due to failure to achieve the necessary depth of sampling by providing sufficient surgical confidence to avoid complications. Surgeons should keep in mind that there is no difference between LP and RP in terms of high degree complication rates and that the surgical attitude does not cause a difference between the groups. Although there are many studies evaluating TUR-BT success rates according to surgical experience in the literature, our study is the first study that showed different surgical attitudes to patient groups.

Ethics

Ethics Committee Approval: Because of the study was designed as retrospective study, ethics committee approval was not obtained.

Informed Consent: Because of the study was designed as retrospective study, informed consent was not taken from the patients.

Peer-review: Externally peer-reviewed.

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Original Article

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Magnetic Resonance Imaging Findings of Multilocular Cystic Renal Cell Carcinoma and Clinical-pathologic Comparison

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Abstract

Objective: Multilocular cystic renal cell carcinoma (RCC) is a unique type of renal carcinoma characterized by multi-loculated cystic masses. The aim of this study was to retrospectively evaluate the magnetic resonance imaging (MRI) findings of multilocular cystic RCC.

Materials and Methods: All patients were examined by MRI. Two radiologists retrospectively evaluated MRI features, and compared radiological findings and Bosniak category with histopathological findings.

Results: The patient population comprised seven men and three women with a mean age of 52.9 years (range:37-61 years). The margins of the multi-loculated cystic masses were well defined in all patients, and there was no sign of infiltration of the adjacent tissue and metastatic lymphadenopathy.

Conclusion: Multilocular cystic RCC exhibits non-malignant behavior and frequently has a long survival. The size of the lesion at diagnosis is variable, but there is no evidence of infiltration and metastasis in patients at diagnosis.

Keywords: Multilocular cystic renal cell carcinoma, MRI, renal cell carcinoma

Introduction

Multilocular cystic renal cell carcinoma (RCC) is a distinctive subgroup of RCC that constitutes approximately 2.3-3.1% of all RCCs (1,2). This group of tumors was listed as a rare variant of RCC by the World Health Organization (WHO) in 2016. Multilocular cystic RCC is a low-grade tumor and prognosis is considered to be favorable compared to conventional clear cell RCC (3), which is the most common RCC subtype. Patients with cystic multilocular RCC who undergo resection have excellent prognosis. The multilocular cystic RCC is considered benign by some pathologists (4) due to the lack of progression or metastasis (5,6).

The imaging finding of multilocular cystic RCC is septated, multilocular, solitary renal cyst with or without solid portions similar to other cystic renal masses. The differential diagnosis of multilocular cystic RCC includes cystic renal cell carcinomas, cystic nephroma, and complicated renal cysts (7).

The aim of this study was to evaluate the magnetic resonance imaging (MRI) characteristics of multilocular cystic RCC, and to present typical and atypical imaging aspects of multilocular cystic RCC.

Materials and Methods

Patients

This study was based on the patients with renal tumors who were investigated by MRI between January 2005 and May 2016, and was performed retrospectively in our institution (the protocol number of non-interventional investigation ethical committee approval was 3476 and decision number was

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2017/25-30). The requirement for informed consent was waived due to the retrospective nature of the study. Of 698 cases with a diagnosis of renal tumor, 10 patients were diagnosed as having multilocular cystic RCC. All patients with multilocular cystic RCC were enrolled in this study. The patients were between the ages of 37-61 and female/male ratio was 3/7. All patients were evaluated with abdominal MRI.

Imaging Methods

All MRI studies were obtained on a 1.5-T MR scanner (Gyroscan Intera, release 8.1; Philips Medical Systems, Best, the Netherlands) using a 4-channel phased-array coil, including routine and post-contrast (0.1 mmol/kg body weight Gadolinium-chelates at 2-2.5 mL/s) acquisitions. MRI was comprised of axial and coronal T2-weighted fast spin-echo images (TR, 519 ms; TE, 120 ms; section thickness, 5 mm; gap, 1 mm; ETL, 77; matrix size, 256x256; field of view, 40.5 cm), axial dual-echo T1-weighted in-phase and opposed-phase gradient-echo images (TR, 154; TE, 2.3-4.6; section thickness, 5 mm; gap, 1 mm; matrix, 256x256; field of view, 40.5 cm), axial spectral fat-saturated T2-weighted fast spin-echo images (TR, 2145 ms; TE, 70 ms; section thickness, 7 mm; gap, 1 mm; ETL, 24; matrix size, 256 x 256; field of view, 40.5 cm), and axial three dimensional frequency-selective fat-saturated T1-weighted gradient-echo images (TR, 316 ms; TE, 5 ms; section thickness, 5 mm; matrix, 512x512; field of view, 40.5 cm). Dynamic contrast-enhanced axial T1-weighted images were performed in the corticomedullary and nephrographic phases after administration of a bolus of 0.1 mmol per kilogram of body weight gadolinium chelates.

Image Interpretation

Two radiologists (M.S., C.A.) evaluated MRI in consensus. To characterize the renal cysts, Bosniak radiological classification was used (8,9,10). Tumor location and size, Bosniak category, tumor shape, contour, regional lymph node metastasis, and presence of tumoral invasion to perirenal fat, sinus, adrenal or renal veins were investigated.

Results

Clinical and Pathological Results

The frequency of multilocular cystic RCC in our study group was 1.4%. The mean age of the patients with multilocular cystic RCC was 52.9 years (range: 37-61 years). Three patients were female and seven were male. There was no evidence of additional systemic malignancy, renal stone disease or congenital renal abnormality in our study group. The tumor sizes ranged from 3.5 cm to 10 cm (mean=6.1 cm).

According to assessment of macroscopic specimens, all patients had cystic renal tumors. These tumors were located in the renal parenchyma and there was no evidence of extension into the renal sinus or local invasion to adjacent tissue or organs in all patients. The diagnosis of multilocular cystic RCC was confirmed histopathologically in all patients. Nine patients were Fuhrman grade 1 and one patient was Fuhrman grade 2. Four patients (40%) had stage T1a, four (40%) had stage T1b, and two (20%) had stage T2. None of the patients had Fuhrman grade 3 or 4, stage T3 or T4, renal vein tumor thrombus or distant metastasis at the time of diagnosis. All cases were N0 and M0. All patients were followed up clinically and radiologically for 36-96 months (mean=77.1 months). During the follow-up, no local recurrence, regional lymph node metastasis, or distant metastasis was observed.

MRI Features

All patients underwent MRI. Because of the cystic part, multilocular cystic RCCs had low signal intensity on T1-weighted images (T1WI) and high signal intensity on T2-weighted images (T2WI). The lesions had well-defined outer margins with thick capsules. After the administration of paramagnetic contrast agent, variable enhancement was observed on the septations and wall of cysts of Bosniak category 3 and 4 cysts. On MRI, the cystic renal lesions were found in five patients on right side and in five patients on the left side. These lesions were located at the upper pole of the kidney in seven patients, at the lower pole in two patients and at the interpolar region in one patient.

All of the renal tumors were in cystic and none of the renal tumors that were diagnosed as multilocular cystic RCC had a complete solid structure. The Bosniak categories of the patients were II in one patient, IIF in one patient, III in six patients and IV in two patients (Figures 1,2,3 and 4). There was no evidence of multifocal or bilateral multilocular cystic RCC. One patient had a chromophobe RCC with Bosniak category II cyst in the same kidney.

In MRI, there were no changes in signal intensity due to hemorrhage or calcification. In our study, a synchronous solid or

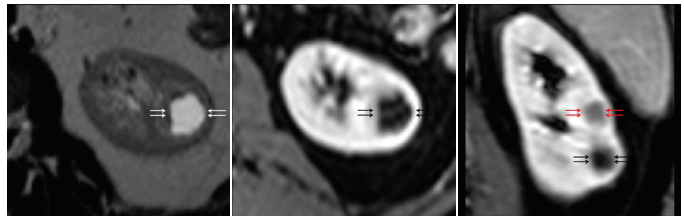

Figure 1. a-c. Bosniak category II cyst; the lesion was detected incidentally during intraoperative ultrasonography for neighboring solid renal tumor (red arrows) and was histopathologically confirmed as multilocular cystic renal cell carcinoma. Axial (a) T2-weighted image shows few small septations in the renal cyst at the lower pole of the left kidney (black arrows). The corticomedullary phase of the dynamic study (b) and late phase post-contrast coronal T1 image (c) show no enhancement of the few septa compared to the renal cortex in the mass (white and black arrows)

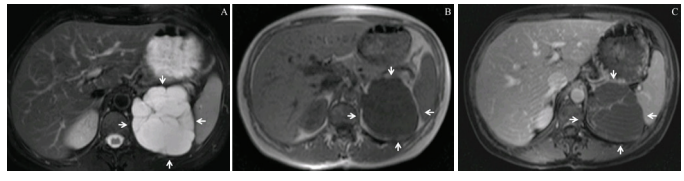

Figure 2. a-c. Bosniak category IIF cyst; after a follow-up period of 1 year, the lesion increased in size and was histopathologically confirmed as multilocular cystic renal cell carcinoma. The axial (a) T2-weighted image shows a giant septated cystic mass at the upper pole of the left kidney. The axial (b) T1-weighted image shows well-demarcated cystic renal mass. The corticomedullary phase of the dynamic study (c) shows mild enhancement in a few septa compared to the renal cortex in the mass

cystic renal neoplasm was found in both kidneys in one patient. The demographic characteristics of patients were comparable in each group with Bosniak category II, IIF, III, and IV lesions. Table 1 provides the MRI, pathological and demographic features of all patients.

Significance of the Study

This study revealed that multilocular cystic renal cell carcinomas are characterized by multi-loculated cystic masses. The Bosniak categories of multilocular cystic renal cell carcinomas were observed II, IIF, III and IV. The differential diagnosis of multilocular cystic renal cell carcinoma includes cystic renal cell carcinomas, cystic nephroma, and complicated renal cysts.

Discussion

Multilocular cystic RCC is a rare subgroup observed predominantly in men with an incidence varying between 1-1.5% of all renal malignancies and generally found in the fifth-sixth decades (11,12,13). In our study, the incidence was similar to previous studies. The clinical symptoms of the patients with multilocular cystic RCC are non-specific and patients are usually asymptomatic (13,14). In practice, multilocular cystic RCC is a coincidentally detected tumor. If totally resected, multilocular cystic RCC has an excellent prognosis. Local recurrence and distant metastasis have not been reported in the English literature (11,14,15).

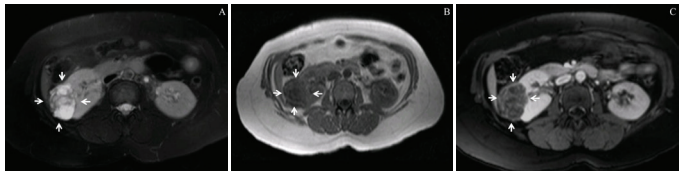

Figure 3. a-c. Bosniak category III cyst; histopathologically confirmed as multilocular cystic renal cell carcinoma. The axial (a) T2-weighted image shows a septated cystic mass with heterogeneous appearance and hypointense rim in the right kidney (arrows). The axial (b) T1-weighted image shows well-demarcated hypointense renal mass (arrows). The corticomedullary phase of the dynamic study (c) shows enhancement in the septa and cyst wall compared to the renal cortex in the mass (arrows)

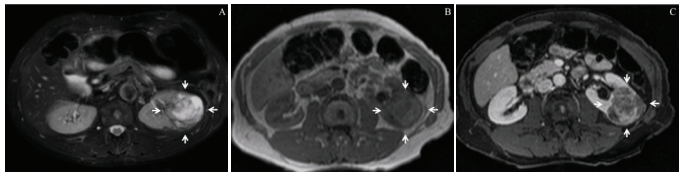

Figure 4. a-c. Bosniak category IV cyst; histopathologically confirmed as multilocular cystic renal cell carcinoma. The axial (a) T2-weighted image shows a heterogeneous cystic mass with solid portion in the left kidney (arrows). On the axial (b) T1-weighted image, the mass has a well-demarcated and hypointense appearance (arrows). The corticomedullary phase of the dynamic study (c) shows prominent enhancement of the septa and solid portion of the mass (asterisk) compared to the renal cortex (arrows)

The multilocular cystic RCC was defined as a rare entity with excellent prognosis according to WHO 2004 criteria. According to WHO, the diagnostic criteria for multilocular cystic RCC are a multilocular cystic appearance, a limited solid component in the small areas with no expansile nodules and no tumor necrosis, and microscopically low Fuhrman grade (16). Histopathologically, these tumors are well demarcated and separated from the kidney by a thick capsule, that may contain fluid, clear cell lining septations, vascularized or non-vascularized fibrosis (1). They consist of multiple fibrous septa composed of malignant epithelial cells with clear cytoplasm (10). In immunohistochemical staining, multilocular cystic RCC is usually positive for vimentin, EMA and CD10 (1).

In MRI, multilocular cystic RCC is defined as septated, variable sized multilocular cystic tumors with fibrous capsule, and they cannot be differentiated from complicated non-malignant renal cysts and other cystic RCC types (1,15). Conventional MRI sequences provide the data of tumor location and internal structure of the multilocular components. The MRI signal of multilocular cystic RCCs depends on the content of the cyst fluid. The fluid portion of multilocular cystic RCC usually appears as hypointense on T1WI and hyperintense on T2WI. Fibrous septations are usually isointense on T1WI and markedly hypointense on T2WI. However, overall or partial portion of the tumor may be observed as hyperintense on T1WI and variable hypointense on T2WI due to intra-tumoral hemorrhage. The signal alteration due to intra-tumoral hemorrhage may contain fluid-fluid level. In our series, the presence of the limited hypointensity on T2WI and the hyperintensity on T1WI was detected in 40% of the patients.

After contrast agent administration in MRI, the enhancement pattern of the tumor is variable, depending on the presence of solid portion and distribution of the cellular component and the fibrous tissue. On contrast enhanced sequences, asymmetric septal, irregular cystic wall or solid part enhancement may be observed. One patient (10%) had Bosniak type II cyst and no

Table 1. Imaging, pathologic and demographic features of the study group

Case	Age	Gender	Side	Location	Bosniak category	Tumor size (cm)	Fuhrman grade	Tumor stage
1	52	M	Left	Upper pole	IV	5.2	2	T1b
2	61	M	Left	Upper pole	IV	5.5	1	T1b
3	60	M	Right	Upper pole	III	5.3	1	T1b
4	59	M	Right	Upper pole	III	3.5	1	T1a
5	50	F	Left	Upper pole	IIF	8	1	T2
6	52	F	Right	Interpolar	III	5.2	1	T1b
7	37	M	Left	Lower pole	III	10	1	T2
8	43	M	Right	Upper pole	III	3	1	T1a
9	54	M	Left	Lower pole	II	2	1	T1a
10	61	F	Right	Upper pole	III	5	1	T1a

M: Male, F: Female

contrast enhancement was observed in the septations. In one patient (10%) with Bosniak type IIF cyst, the septations of the tumor showed tiny enhancement in our study. The remaining eight patients (80%) have asymmetric septal and irregular cystic wall enhancement in MRI compatible with Bosniak type III and IV cysts. Furthermore, enhancement of the solid portion was observed in two patients (20%) with Bosniak type IV cyst.

For the first time, the renal cysts were classified by Bosniak in 1986 (7). Bosniak revised his classification in 1997 and 2012 (8,9). According to the Bosniak classification, renal cysts are divided into 4 groups. Bosniak I, II and IIF cysts contain benign features. Bosniak III cysts are complicated cystic lesions with septal enhancement and thickening. Bosniak IV cysts are clearly malignant lesions and contain solid portion. The distribution of the Bosniak category in multilocular cystic RCC ranged from type IIF cyst to type IV cystic tumor. Similar to previous studies, multilocular cystic RCCs tend to be Bosniak type III cyst (60) and to have a multilocular appearance in our study.

Patients usually present with non-specific symptoms similar to other types RCC, such as low back pain and hematuria (13). In our study, tumor size, gender distribution, clinical symptoms, tumor lateralization, Fuhrman grade and Tumour, Node, Metastasis stage were found to be similar with the literature (11,14,17). Complete resection of the renal cyst was performed in all patients. In six patients, cystic tumors were resected by nephron sparing surgery and radical nephrectomy was performed in the remaining four patients. Nephron-sparing surgery may be a preferable treatment method in patients with multilocular cystic RCC, especially in Bosniak type 3 cysts (18). During the follow-up period, all patients were uneventful in our study.

Study Limitations

Our study has some limitations. First, this is a retrospective study performed in renal carcinoma patients who were met during the diagnostic procedures or somehow discussed in multidisciplinary meetings; hence the selection bias is inevitable. Second, the study reflects the results of a single institute; larger series may be achieved in multi-institutional studies. However, our study group consisted of patients with images in 12-year PACS archive and with close clinical and radiological follow-up.

Conclusion

Multilocular cystic RCC is a rare malignant renal tumor and should be kept in mind in the differential diagnosis of complicated cystic renal masses. In our study, multilocular cystic RCCs appeared as complicated cystic lesions and were identified as Bosniak II-IV cysts in MRI.

Ethics

Ethics Committee Approval: Because of the study was designed as a retrospective study, ethics committee approval was not obtained.

Informed Consent: Because of the study was designed as a retrospective study, informed consent was not taken from the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: C.A., Design: C.A., Data Collection or Processing: O.B., Ö.D., G.A., B.T., K.Y., Analysis or Interpretation: C.A., M.S., Literature Search: O.B., Ö.D., G.A., B.T., K.Y., Writing: C.A.

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

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Original Article

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The Effect of Framingham Score on the Oncological Outcomes in Localized (T1-T2 Stage) Renal Cell Carcinoma Patients

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Abstract

Objective: To evaluate the effect of cardiovascular disease risk on local recurrence, distant metastasis development and cancer-specific survival in patients with localized (stage 1 and 2) renal cell carcinoma (RCC).

Materials and Methods: Data of patients who underwent partial or radical nephrectomy due to pathological stage 1 and 2 RCC between September 2009 and July 2016 were retrospectively evaluated. Ninety-six patients with fully accessible data were included in the study. Demographic data, histological tumor type, Fuhrman grading, local recurrence, metastasis and survival after nephrectomy were recorded. Framingham risk score, which predicts cardiovascular disease within 10 years, was calculated in all patients. The patients were divided into three groups as low (group 1), moderate (group 2) and high risk (group 3).

Results: Mean age of patients was 58.66±10.55 years at the time of nephrectomy. Nine (9.4%) patients had local recurrence, 12 (12.5%) had distant metastasis and 11 (11.5%) died due to cancer during a median follow-up period of 57 (6-102) months. Regarding intergroup comparison, local recurrence rate (21.9%, p=0.012) and distant metastasis rate (25%, p=0.025) were significantly higher in group 3, and predicted recurrence-free survival (66.4 months, p=0.005), metastasis-free survival (77 months, p=0.017) and cancer-specific survival (79.9 months, p=0.024) were found to be significantly lower. In univariate analysis, body mass index, total cholesterol level, estimated glomerular filtration rate and Framingham risk score were independent predictive factors for local recurrence, distant metastasis development and cancer-specific survival. In multivariate analysis, body mass index, estimated glomerular filtration rate and Framingham risk score were more significant.

Conclusion: Patients who are at high risk of developing cardiovascular disease have more local recurrence, distant metastasis and cancer-specific mortality rates, even though nephrectomy is performed due to localized RCC. Therefore, we suggest that these patients should be followed more carefully in the post-nephrectomy period.

Keywords: Cardiovascular disease risk, Framingham risk score, nephrectomy, oncologic outcomes, renal cell carcinoma

Introduction

Renal cell carcinoma (RCC) with increased rates of incidental detection during the localized stage (stage 1-2) with a small size accounts for 2-3% of all cancers (1). Its incidence increases in the sixth and seventh decades, and known predisposing factors are smoking, obesity and hypertension (2).

Although the presence of tumor-related anatomical and histological factors [tumor-node-metastasis (TNM) stage, Fuhrman tumor grade, histological type, tumor size, presence of necrosis, etc.] and patient-related factors (clinical signs, symptoms, general health status, laboratory findings, molecular factors) is known (3), the importance of new molecular markers continues to be investigated with current studies (4,5,6). In localized RCC patients, local recurrence or distant metastasis rates after partial or radical nephrectomy have been reported to be 20-40% (7). The effects of presence and components of metabolic syndrome on oncologic outcomes in localized RCC have been investigated in many studies. The common belief in these studies is that the metabolic syndrome is a poor prognostic factor for RCC, that it increases the incidence of RCC approximately 4-6 times, leads to an increase in tumor size and stage, and significantly reduces progression-free survival (PFS) (8,9,10). Hypertension was found to be the worst prognostic risk factor in the publications investigating the effects of

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individual metabolic syndrome components on oncologic outcomes in RCC (11,12). However, there are no studies in the literature that predict post-nephrectomy outcomes according to developing 10-year cardiovascular disease risk.

In our study, we aimed to investigate the effect of cardiovascular disease risk, calculated according to Framingham score before nephrectomy, on the local recurrence, distant metastasis and cancer-related mortality rates in patients with pathologic stage 1-2 RCC.

Materials and Methods

We retrospectively evaluated 148 patients who underwent partial or radical nephrectomy due to localized RCC, and whose pathological diagnosis was stage 1 or 2 RCC according to TNM classification in our clinic between September 2009 and July 2016. The demographic data of the patients, histological tumor type, Fuhrman grading, presence of necrosis, tumor side, localization, size, type of surgery, follow-up period after nephrectomy, local recurrence, metastasis and survival rates were recorded. Estimated glomerular filtration rate (eGFR), calculated by the short-term Modification of Diet in Renal Disease (MDRD) formula using preoperative creatinine, age, gender and race, was recorded.

Framingham Risk Score

The Framingham risk score was prepared according to long-term studies of National Cholesterol Education Program Adult Treatment Panel 3 (NCEP ATP 3) and National Heart, Lung and Blood Institute and it is based on research in 1976. It was first tried in 1998 in daily practice and it is used to estimate the 10-year cardiovascular (myocardial infarction, coronary death, angina, etc.) risk of an individual. Reliability and validity have been provided by various studies (13). The Framingham score, which is one of the most commonly used risk calculations, systematically predicts the risk of cardiovascular disease and related mortality by systematic mathematical equations (14). The aim of this risk score is to determine measurable and preventable risk factors that can affect the development of cardiovascular disease, to provide lifestyle and behavior change in patients at risk and to determine appropriate treatment.

The Framingham risk calculator, developed for patients between the ages of 30-74, only calculates 10-year cardiovascular event risk (total of non-fatal and fatal coronary events). The parameters used in the Framingham risk score include risk factors associated with coronary heart disease, such as age, gender, blood pressure, total cholesterol, high density lipoprotein (HDL) levels, smoking, and diabetes. Scoring is performed for each parameter and the total score is calculated. The percentages that correspond to the specified score range refer to the 10-year risk of developing cardiovascular disease separately for men and women. According to this, <10% indicates a low-risk, 10-20 % a moderate-risk and >20% a high risk (15). This risk score is both easy to implement and does not require additional invasive intervention or cost because the necessary data can be easily obtained in clinical practice.

The data required to calculate Framingham risk score of patients included in the study, which include age, gender, total cholesterol, HDL level, systolic blood pressure, use of antihypertensive treatment, smoking, and diabetes, were obtained from hospital archive and patient information system. These data were used in the calculation of Framingham score in the week immediately preceding nephrectomy. Ninety-six patients with complete data were included in the study without randomization. According to the Framingham risk score, the patients were divided into three groups as low-risk (<10%), moderate-risk (10-20%) and high risk (> 20%) respectively, and were named as group 1, 2 and 3, respectively. Three groups were compared in terms of oncologic outcomes.

Statistical Analysis

To compare the differences between the three groups, Pearson chi-square was used for categorical variables, One-way analysis of variance (ANOVA) or Kruskal-Wallis test were used for continuous variables. Tukey or Dunn-Bonferroni tests were applied for multiple comparisons. Kaplan-Meier was used for survival analysis and Cox regression analysis was used to determine the variables that affect this. Spearman test was used for correlation analysis. Analysis was performed using IBM SPSS Statistics 21 (IBM, Armonk, NY USA) software. $p<0.05$ was considered statistically significant.

Results

The mean age of the 96 patients included in the study was 58.66±10.55 years, and 56 (58.3%) were male and 40 (41.7%) were female. During the median follow-up period of 57 (6-102) months, nine (9.4%) patients had local recurrence, 12 (12.5%) had distant metastasis and 11 (11.5%) died due to cancer. Distant metastases were seen in lung in six patients, bone in two patients and liver in four patients. Demographic, pathological, clinical data and oncologic outcomes of the patients are shown in Table 1.

Regarding intergroup comparisons, local recurrence rate (21.9%, p=0.012) and distant metastasis rate (25%, p=0.025) were significantly higher in group 3 (Table 1). The predicted recurrence-free survival in group 3 (66.4 months) was significantly lower than in group 1 (98.9 months) and group 2 (99.2 months) (p=0.021 and p=0.010, respectively). No significant difference was observed between the predicted recurrence-free survivals of the patients in group 1 and group 2 (p=0.935) (Table 2, Figure 1).

The predicted metastasis-free survival in group 3 (77 months) was significantly lower than in group 1 (92.2 months) (p=0.013). There was no significant difference between survival in group 2 (94.5 months) and group 1 and group 3 patients (p=0.404 and p=0.061, respectively) (Table 2, Figure 2).

The predicted cancer-specific survival in group 3 (79.9 months) was significantly lower than in group 1 (102 months) (p=0.007). There was no significant difference between predicted cancer-specific survival in group 2 (94.7 months) and group 1 and group 3 (p=0.401 and p=0.128, respectively) (Table 2, Figure 3).

In the univariate analysis, body mass index (BMI), total cholesterol level, eGFR and Framingham risk score were independent predictive factors for local recurrence, distant metastasis and cancer-specific survival. In multivariate analysis,

Table 1. Demographic, pathological, clinical data and oncologic outcomes of patients					
Parameters	**Group 1 (n=31)**	**Group 2 (n=33)**	**Group 3 (n=32)**	**Total (n=96)**	**p**
Age, mean ± standard deviation	54.84±11.17[a]	61.30±9.32[b]	59.63±10.39[ab]	58.66±10.55	† 0.039*
Gender (n,%) Male Female	 17 (54.8) 14 (45.2)	 19 (57.6) 14 (42.4)	 20 (62.5) 12 (37.5)	 56 (58.3) 40 (41.7)	‡ 0.822
BMI (kg/m^2) (median, 25th -75th percentile)	23.3 (21.3-24.4)[a]	23.6 (21.9-26.2)[a]	27.6 (24.5-29.0)[b]	24.2 (22.3-26.8)	§ 0.226/<0.001/<0.001*
Smoking Yes No	 13 (41.9) 18 (58.1)	 22 (66.7) 9 (27.3)	 25 (78.1) 9 (21.9)	 60 (62.5) 36 (37.5)	‡ 0.016*
Hypertension Yes No	 1 (3.2) 30 (96.8)	 9 (27.2) 24 (72.8)	 17 (53.1) 15 (46.9)	 27 (28.1) 69 (71.9)	‡ <0.001*
Diabetes Yes No	 3 (9.7) 28 (90.3)	 5 (15.2) 28 (84.8)	 15 (46.9) 17 (53.1)	 23 (23.9) 73 (76.1)	‡ 0.001*
Surgery Radical Partial	 20 (64.5) 11 (35.5)	 23 (69.7) 10 (30.3)	 25 (78.1) 7 (21.9)	 68 (70.8) 28 (29.2)	‡ 0.486
Tumor side Right Left	 14 (45.2) 17 (54.8)	 17 (51.5) 16 (48.5)	 15 (46.9) 17 (53.1)	 46 (47.9) 50 (52.1)	‡ 0.87
Tumor localization Upper pole Middle pole Lower pole Hilum	 9 (29) 8 (25.8) 12 (38.7) 2 (6.5)	 7 (21.2) 8 (24.2) 14 (42.5) 4 (12.1)	 10 (31.3) 6 (18.8) 7 (21.9) 9 (28)	 26 (27) 22 (22.9) 33 (34.3) 15 (15.8)	‡ 0.209
Pathological tumor size (cm) (median, 25th -75th percentile)	4.4 (3.2-5.5)	4.6 (2.7-6)	4.5 (3-5.8)	4.4 (3.02-5.95)	§ 0.98
Histological subtype, (n,%) Clear cell Papillary Chromophobe Other	 22 (71) 4 (12.9) 3 (9.7) 2 (6.4)	 25 (75.8) 4 (12.1) 2 (6.1) 2 (6.1)	 23 (71.9) 6 (18.8) 1 (3.1) 2 (6.2)	 70 (72.9) 14 (14.5) 6 (6.3) 6 (6.3)	‡ 0.899
Fuhrman grade (n,%) 1-2 3-4	 18 (58) 13 (42)	 22 (66.7) 11 (33.3)	 22 (68.8) 10 (31.2)	 62 (64.5) 34 (35.5)	‡ 0.644
Pathological stage (n,%) T1a T1b T2a-T2b	 13 (41.9) 16 (51.6) 2 (6.4)	 15 (45.5) 13 (39.4) 5 (15.2)	 17 (53.1) 11 (34.4) 4 (12.5)	 45 (46.8) 40 (41.7) 11 (11.5)	‡ 0.604
TNM stage (n,%) Stage 1 Stage 2	 29 (93.5) 2 (6.5)	 28 (84.8) 5 (15.2)	 28 (87.5) 4 (12.5)	 85 (88.5) 11 (11.5)	‡ 0.537
Presence of necrosis (n,%) Yes No	 4 (12.9) 27 (87.1)	 9 (27.3) 24 (72.7)	 7 (21.9) 25 (78.1)	 20 (20.8) 76 (79.2)	‡ 0.362
eGFR (median, 25th -75th percentile)	95.46 (78.98-108.56)[a]	81.96 (69.27-91.38)[b]	78.97 (72.44-89.01)[b]	83.25 (73.23-97.88)	§ 0.007/0.003/0.637*
Follow-up period, median (min-max) month	59 (13-102)	57 (14-102)	50 (6-100)	57 (6-102)	§ 0.571
Local recurrence rate (n,%)	1 (3.2)	1 (3.0)	7 (21.9)	9 (9.4)	‡ 0.012*
Distant metastasis rate (n,%)	1 (3.2)	3 (9.1)	8 (25)	12 (12.5)	‡ 0.025*
Cancer-specific survival rate (%)	96.8	90.9	78.1	88.5	‡ 0.059

a, b, c: Groups with statistically significant differences were shown with different letters
There is no statistical difference between the groups indicated by the same letter.
ab: Group with no statistically significant difference from other two groups
† ANOVA ‡ Chi-square § Kruskal-Wallis * p <0.05 (There is a significant difference between groups)
BMI: Body mass index, TNM: Tumour-node-metastasis,
eGFR: Estimated glomerular filtration rate

BMI, eGFR and Framingham risk score were found to be more significant (Table 3). In addition, according to Spearman correlation analysis, a significant negative correlation was found between eGFR and Framingham risk score (r=-0.380, p <0.001) (Figure 4).

Discussion

The tumor-related anatomical and histological factors affecting prognosis in RCC are TNM stage, Fuhrman tumor grade, RCC histological subtype, and tumor size. Nowadays, many nomograms and models have been defined for the development of recurrence and progression in both localized and metastatic disease before and after nephrectomy. The most important of these models as independent prognostic factors are TNM stage, Fuhrman degree and patient performance status.

The pathologic tumor stage in RCC is the most important prognostic factor alone, and the 5-year survival rate in T1-2N0M0 is 70-90% (16). The 10-year cancer-specific survival rates for pathological stage T1a, T1b, T2 are 90-95%, 80-85% and 75%, respectively (17). Large-sized, organ-confined tumors have been found to have a greater degree of clear cell tumor histology and a higher grade of Fuhrman (18).

In a multicentre study involving 5332 patients, the 5-year cancer-specific survival rates reported by Novara et al. (19) were 94.9%, 92.6%, 85.4% and 70% for pT1a, pT1b, pT2a, pT2b, respectively. In a current study involving T1, T2 and T3a patients, local or distant recurrence was 21.57% and cancer-specific survival was 78.43% at 50.8±18.1 months follow-up (20).

In RCC, the Fuhrman nuclear grade revealed a link between tumor stage, size, nodal involvement and systemic metastasis (21). When all pathological stages were compared, 5-year

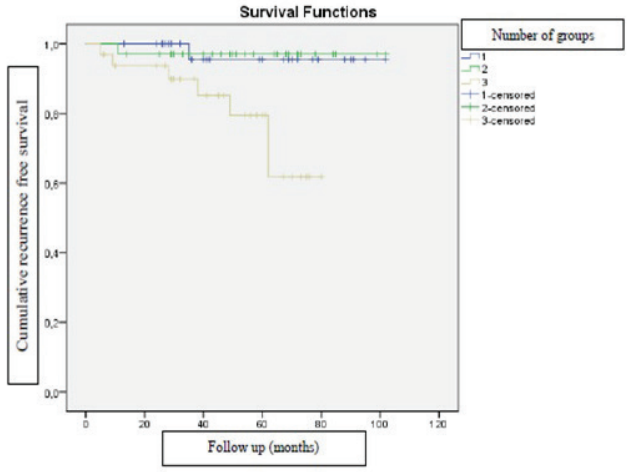

Figure 1. Graph of Kaplan-Meier analysis for predicted recurrence-free survival in three groups

Table 2. Predicted recurrence-free, metastasis-free and cancer-specific survival for three groups

	Mean	% 95 CI		Median	% 95 CI		p
		Lower	Upper		Lower	Upper	
Predicted recurrence-free survival time (month)							
Group 1	98.9	93.1	104.7	-	-	-	***0.005***
Group 2	99.2	93.9	104.5	-	-	-	
Group 3	66.4	57.9	75.0	-	-	-	
Total	93.4	88.1	98.6	-	-	-	
Predicted metastasis-free survival time (month)							
	Mean	% 95 CI		Median	% 95 CI		p
		Lower	Upper		Lower	Upper	
Group 1	92.9	86.9	97.5	-	-	-	***0.017***
Group 2	94.5	86.4	102.5	-	-	-	
Group 3	77.0	63.4	90.6	-	-	-	
Total	90.7	84.7	96.6	-	-	-	
Predicted cancer specific survival time (month)							
	Mean	% 95 CI		Median	% 95 CI		p
		Lower	Upper		Lower	Upper	
Group 1	102.0	102.0	102.0	102.0	-	-	***0.024***
Group 2	94.7	86.8	102.5	-	-	-	
Group 3	79.9	67.2	92.6	-	-	-	
Total	92.2	86.2	98.2	102.0	72.6	131.3	

CI: Confidence interval
Kaplan-Meier (Log-Rank)/The binary difference between the groups was calculated with "Pairwise over strata".

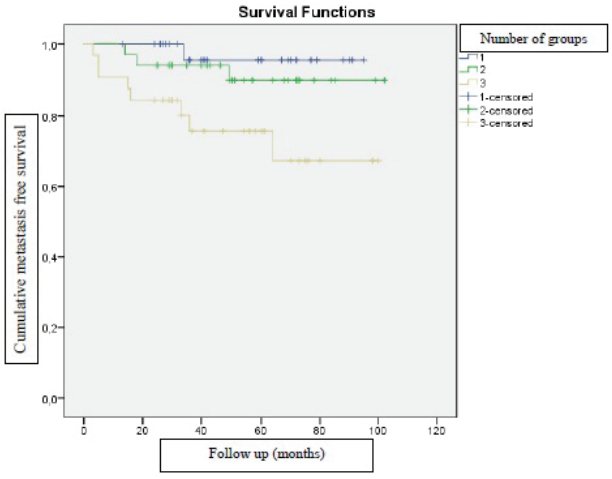

Figure 2. Graph of Kaplan-Meier analysis for predicted metastasis-free survival in three groups

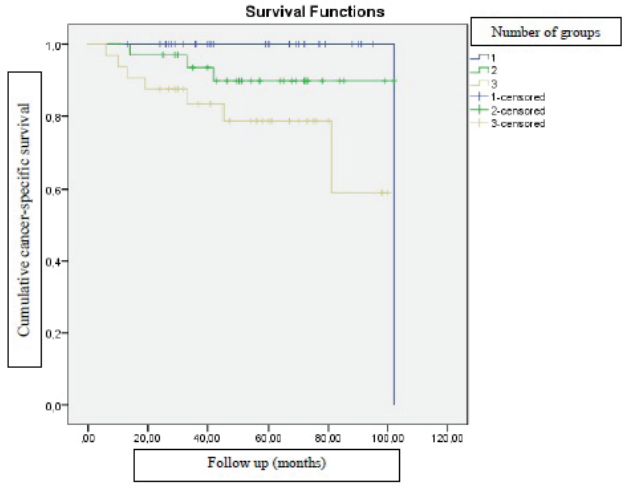

Figure 3. Graph of Kaplan-Meier analysis for predicted cancer-specific survival in three groups

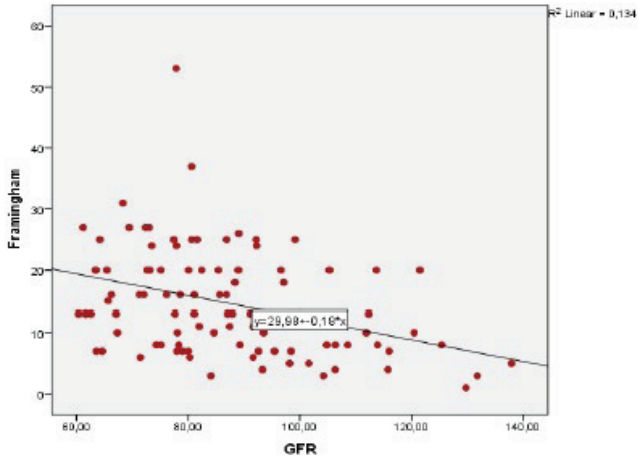

Figure 4. Graphical representation of the correlation between Framingham risk score and eGFR: Estimoted glomenular filtnotion note

survival rates for Fuhrman grades I, II, III, IV were reported as 64%, 34%, 31% and 10%, respectively, and this grading is known to be an important prognostic factor in organ-confined localized disease (22). As a matter of fact, in a multicentre study involving 5009 cases, local recurrence rates at 5 years after nephrectomy were observed as 17.1%, 23.9%, 11.3% and 4.2% for T1a, T1b, T2a and T2b, respectively, during median follow-up of 105 months, and recurrence rates have been reported to be higher in Fuhrman grade 3-4 cases (23).

During a median follow-up of 64 (6-102) months in T1a and T1b patients in our study, local recurrence rates were 9.75% and 14.28%, distant metastasis rates were 9.75% and 25%, and cancer specific survival rates were 92.5% and 86.35%, respectively. Our oncologic results for T1 stage are consistent with current literature data. However, we could not make a significant survival analysis for T2 stage since there were 11 patients and there was no cancer-related mortality during the median 59 (13-99) months follow-up. During the median 57 (6-102) months follow-up of all patients in the T1 and T2 stages, local recurrence was 9.4%, distant metastasis was 12.5%, and cancer-specific survival was 88.5%. Although we observed that Fuhrman grade 3-4 was an independent prognostic factor affecting both local recurrence and cancer-specific survival, we could not find a significant effect on the development of metastasis.

In the literature, there are many studies investigating the effect of metabolic syndrome, including impaired glucose tolerance /diabetes, obesity, high triglyceride levels, low HDL levels and hypertension on oncologic outcomes in RCC (8,9,24). Although there are some contradictory results, metabolic syndrome is thought to be a poor prognostic factor for RCC. It is known that the incidence of RCC increases approximately 4-6 times in patients with three or more metabolic syndrome components (8). It was observed that the tumor size and grade were significantly higher in the presence of metabolic syndrome and that there was a correlation between individual hypertension, diabetes and high triglyceride levels with tumor aggressiveness (9).

Kriegmair et al. (10) showed no significant individual effect of diabetes, obesity (BMI>30 kg/m^2), hypertension and hypertriglyceridemia on progression-free survival (PFS) in localized RCC. However, in the presence of metabolic syndrome consisting of all these components, it was observed that PFS was significantly shortened and cancer-specific survival did not change. When Kocher et al. (11) examined the components of the metabolic syndrome, they found that hypertension has the most significant relationship with high tumor stage, high Fuhrman grade, increased tumor size, increased nephrometry score and non-clear cell histological subtype in RCC.

Eskelinen et al. (12) found a significant relationship between the presence of hypertension and dyslipidemia in patients with local advanced stage RCC at the time of diagnosis and found that, among the metabolic syndrome components, only hypertension was an independent risk factor that increases cancer-related mortality (12). In accordance with these results, another study reported that the presence of type 2 diabetes alone was not found to be a negative prognostic factor for RCC

(25).

When the literature is examined, it is seen that both the presence of metabolic syndrome and the individual components are investigated on the oncologic outcomes in localized RCC. In patients with no required lifestyle changes or medical treatment for blood pressure, lipid profile and body mass index, it is known that they have a risk of developing cardiovascular disease in 10-year follow-up as a result of the cumulative effect of the risk factors. We could not find any study investigating the oncologic outcomes of localized RCC patients classified according to this risk analysis during follow-up after nephrectomy.

Numerous nomograms and risk analyzes are available to estimate the risk of cardiovascular disease, with Framingham Heart Study results affecting most of them (26). The common goal of these risk analyzes is to quantitatively calculate the measurable and preventable risk factors on the development of cardiovascular disease. In this way, it is aimed to determine the appropriate treatment by changing the life style and behavior in the patients at risk.

Smoking, obesity and hypertension are the most important predisposing factors in RCC and are associated with a higher incidence of cancer. Although obesity is known to increase the incidence of RCC, in some studies, better oncologic outcomes have been reported during follow-up after nephrectomy in patients with high BMI (20,27). In our study, although only three patients were in the obese category (BMI ≥30 kg/m^2), we observed that the increase in BMI was associated with more recurrence, distant metastasis and cancer-related mortality, and BMI values were significantly higher in group 3.

Although the number of cigarettes smoked per day and duration of smoking directly affect RCC development, the incidence of RCC decreases by 30% 10 years after smoking cessation (20). In our study, although smoking did not seem to affect oncologic outcomes in univariate and multivariate models, the smoking rate, which is a component of Framingham score, was significantly higher in group 3 where worse prognostic

Table 3. Predictive factors for local recurrence development, distant metastasis development and cancer-specific survival

	Univariate Model				**Multivariate Model**			
Development of local recurrence	HR	%95 CI		**p**	HR	%95 CI		**p**
		Lower	Upper			Lower	Upper	
BMI	1.877	1.381	2.552	***<0.001***	1.779	1.161	2.725	***0.008***
Hypertension	1.118	1.055	1.185	***<0.001***	-	-	-	-
Total cholesterol	1.023	1.009	1.038	***0.001***	-	-	-	-
HDL	0.878	0.774	0.995	***0.042***	-	-	-	-
Fuhrman grade 3-4	3.902	1.560	9.756	***0.004***	5.049	1.388	18.363	***0.014***
eGFR	0.942	0.891	0.995	***0.033***	0.932	0.866	1.003	***0.044***
Framingham risk score	1.192	1.092	1.301	***<0.001***	1.192	1.092	1.235	***<0.001***
	Univariate Model				**Multivariate Model**			
Development of metastasis	HR	%95 CI		**p**	HR	%95 CI		**p**
		Lower	Upper			Lower	Upper	
BMI	1.755	1.364	2.258	***<0.001***	1.755	1.364	2.258	***<0.001***
Hypertension	1.067	1.020	1.117	***0.005***	-	-	-	-
Total cholesterol	1.023	1.010	1.035	***<0.001***	-	-	-	-
eGFR	0.932	0.885	0.981	***0.007***	0.947	0.899	0.998	***0.043***
Framingham risk score	1.125	1.066	1.187	***<0.001***	1.074	0.998	1.156	***0.042***
	Univariate Model				**Multivariate Model**			
Cancer spesific survival	HR Lower	%95 CI		**p**	HR Lower	%95 CI		**p**
		Upper				Upper		
BMI	2.161	1.563	2.989	***<0.001***	2.161	1.563	2.898	***<0.001***
Hypertension	1.076	1.029	1.125	***0.001***	-	-	-	-
Presence of diabetes	3.716	1.055	13.093	***0.041***	-	-	-	-
Total cholesterol	1.019	1.006	1.033	***0.004***	-	-	-	-
Fuhrman grade 3-4	2.788	1.209	6.429	***0.016***	-	-	-	-
eGFR	0.930	0.879	0.984	***0.012***	0.905	0.816	1.003	***0.042***
Framingham risk score	1.139	1.076	1.205	***<0.001***	1.087	1.020	1.159	***0.011***

CI: Confidence interval, HR: Heart rate, BMI: Body mass index, HDL: High-density lipoprotein, eGFR: Estimated glomerular filtration rate
Cox Regression Analysis

outcomes were observed. The incidence of hypertension and diabetes was also significantly higher in this high-risk group.

When all patients in our study were divided into groups according to Framingham risk score, local recurrence rate (21.9%) and distant metastasis rate (25%) were significantly higher, and predicted recurrence-free survival (66.4 months), metastasis-free survival (77 months) and cancer-specific survival (79.9 months) were significantly lower in group 3 with a high risk of developing cardiovascular disease. Although the cancer-specific survival rate was lower (78.1%) in the high-risk group, it was not statistically significant (p=0.059).

As known, partial nephrectomy technique has gained significant role in small renal masses (especially in T1 stage) based on the idea that nephron loss after nephrectomy may increase the course of chronic kidney disease (CKD). An eGFR value of 45-60 mL/min/1.73 m^2, which is the third stage CKD indicator, was observed in 65% after radical nephrectomy and 20% after partial nephrectomy. The rate of severe CKD (eGFR<45 mL/min/1.73 m^2) was 36% after radical nephrectomy and 5% after partial nephrectomy (28). It is known that the decrease in eGFR after nephrectomy leads to an increase in cardiovascular disease and mortality, and a decrease in overall survival (29,30). Ahmedov et al. (20) demonstrated that pre-operatively lower eGFR values also adversely affected cancer-specific survival and recurrence-free survival. In our study, preoperative eGFR was > 60 mL/min/1.73 m^2 in all patients, however, significantly lower eGFR values were found in group 3 with a high risk of cardiovascular disease within 10 years and these patients had worse oncologic outcomes during follow-up. In univariate and multivariate analyzes, we observed that preoperative eGFR level affected local recurrence, metastasis rates and cancer-specific survival. In accordance with these findings, we also showed a significant negative correlation between eGFR and Framingham risk score (r=-0.380, p<0.001). This suggests that relatively lower preoperative eGFR is an independent factor that adversely affects overall survival by increasing both RCC-related mortality and cardiovascular risk.

Limitations of the Study

The retrospective design of our study, the low number of patients, the lack of randomization, and the fact that the follow-up results belong to a single center are the main limiting factors.

Conclusion

In patients with localized-stage RCC who are at high risk of developing cardiovascular disease, more local recurrence, distant metastasis and cancer-related mortality rates can be observed postoperatively despite curative treatment with nephrectomy. Therefore, we suggest that these patients should be followed more carefully in the post-nephrectomy period. The results should be supported with prospective, randomized, multicentre, large-scale studies with longer follow-up periods and the issue should be further clarified.

Ethics

Ethics Committee Approval: Ethical committee approval was not obtained since it is a retrospective study.

Informed Consent: Patients were pre-operatively informed about the use of oncologic follow-up data such as recurrence, metastasis development and survival analysis in various oncological studies without revealing patient names and identity information. The data of patients who did not consent were not used.

Peer-review: Externally and Internally peer-reviewed.

Author Contributions

Surgical and Medical Application: H.B., Concept: H.B., Design: İ.S., Data Collection or Processing: İ.S., Analysis or Interpretation: İ.S., H.B., Literature Search: İ.S., Writing: İ.S.

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

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Review

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Immunotherapy in Prostate Cancer

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Abstract

In recent years, immunotherapy has become an important treatment alternative in the treatment of many cancers. Research on immunotherapy in prostate cancer has been accelerated by obtaining Food and Drug Administration (FDA) approval of sipuleucel-T for asymptomatic or minimal symptomatic metastatic castration-resistant prostate cancer (CRPC). Despite all these developments, the patients in whom these agents should be used, sequential use and combination strategies remain unclear. In this review, mechanisms of action and survival outcomes of different immunotherapeutic agents and therapeutic cancer vaccines in mCRPC are discussed.

Keywords: Prostate cancer, immunotherapy, vaccine, checkpoint blockers, survival

Introduction

In recent years, treatment alternatives for metastatic castration-resistant prostate cancer (MCRPC) have significantly increased and nowadays, many agents that have been proven to prolong overall survival in this population have been introduced. In addition to docetaxel, which is the backbone of the MCRPC chemotherapy, cabazitaxel provides an additional conventional approach. New generation antiandrogens targeting androgen inhibition such as enzalutamide and abiraterone offer a better toxicity profile. Radium-223 is aradiopharmaceutical and a unique option for patients with symptomatic bone metastasis.

The Food and Drug Administration (FDA) approval of sipuleucel-T in asymptomatic or minimally symptomatic MCRPC initiated the modern era of cancer immunotherapy.

In the progressive process, persistent improvements in survival with checkpoint blockers in patients with different solid tumors resulted in a change in treatment practices. However, although the response rates and survival benefits of checkpoint blockers in prostate cancer have been inadequate so far, the symptoms associated with clinical benefit suggest that these agents should not be abandoned. Strategic patient selection and tactical combination approaches can be a key to unlock immunotherapy in this disease.

Checkpoint Inhibitors

CTLA-4 Inhibitors

Ipilimumab is the first checkpoint inhibitor approved by the FDA in 2011. Ipilimumab is an antibody that blocks cytotoxic T-lymphocyte antigen-4 (CTLA-4) and showed remarkable improvement in overall survival in advanced stage melanoma (1,2). This drug, a complete human IgG monoclonal antibody, inhibits the binding of B-7 on antigen presenting cells (APC) with CTLA-4. Inhibition of CTLA-4/B-7 interaction reveals T cell activation and proliferation. Early ipilimumab clinical trial data from the MCRPC caught a glimpse of clinical activities in this population and provided a justification for additional research.

The first study evaluating Prostat specific ontigen (PSA) modulation and efficacy of ipilimumab in MCRPC was reported by Small et al. (3). As a result of this monotherapy pilot study, a decrease in PSA >50% was observed in two patients for 135 days and 60 days, respectively. A decrease in PSA <50% was reported in the remaining eight patients. Although PSA response is not a good indicator of radiographic response and clinical benefit, these improvements suggested that further evaluation of ipilimumab is needed.

There are two large phase III studies evaluating the effect of ipilimumab on survival in the MCRPC. In the first study, 799 patients with docetaxel-resistant prostate cancer and at least one bone metastasis were divided into 10 mg/kg ipilimumab and placebo groups after radiotherapy (4). The primary outcome of the study was overall survival (OS). In the ipilimumab arm, OS was 11.2 months and 10 months in the placebo arm (HR: 0.85, 95% CI: 0.72-1.00; p=0.053). Although this study did not meet the primary outcome, there was no OS benefit in the post-hoc subgroup analyzes with poor prognostic factors in patients with visceral metastasis, high alkaline phosphatase or low hemoglobin levels, whereas OS benefit was found in the good prognostic group (p=0.0038). This post-hoc analysis result

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contributed to evidence that patients with good prognostic factors would benefit more from immunotherapy (5,6,7).

In another phase III study, non-chemotherapy-treated asymptomatic or minimally symptomatic MCRPC patients without visceral metastasis were randomized to ipilimumab and placebo groups in a two: 1 ratio (8). Overall survival, the primary outcome, did not show a statistically significant difference between the two groups. The median OS was 28.7 months in the ipilimumab arm and 29.7 months in the placebo arm (HR: 1.11; 95% CI: 0.88-1.39; p=0.3667). However, progression-free survival (PFS) was 5.6 months in the ipilimumab arm and 3.8 months in the placebo arm (HR: 0.67; 95.87% CI: 0.55-0.81), while the PSA response was 23% in the ipilimumab arm and 8% in the placebo arm. Significant toxicities were identifiedand the most common side effects associated with the treatment were diarrhea, rash, itching, fatigue, nausea/vomiting and decreased appetite. In the ipilimumab arm, nine treatment-related deaths were reported, while no death was observed in the placebo arm. It is emphasized that this situation requires further research.

Tremelimumab, another anti-CTLA-4 agent, was evaluated in clinical trials of patients with different solid organ tumors. In 11 patients with prostate cancer with PSA recurrence, safety and PSA kinetics were evaluated following short-term androgen suppression treatment with tremelimumab (9). In this small population study, no change in PSA was observed. However, in three patients who had been on tremelimum for months following treatment, prolonged PSA doubling time was observed immediately after two doses. Although the PSA response with CTLA-4 inhibitors is interesting, further analysis is needed because of the hopeless results and the accompanying toxicity with ipilimumab monotherapy in prostate cancer.

PD-1/PDL-1 Inhibitors

FDA-approved PD1/PDL-1 inhibitors, nivolumab, pembrolizumab, durvalumab, atezolizumab, and avelumab have so far been less pronounced in prostate cancer compared to the impressive results in other solid organ tumors.

In one of the first studies evaluating nivolumab in solid tumors including 17 prostate cancer patients, no objective response was reported (10). In the phase Ib study in which pemrolizumab was evaluated in 23 patients with MCRPC and PDL-1 expression level >1%, partial response was observed in only three patients (11). The median response time was 59 weeks (28-62 weeks) and the overall response rate was 13% (95% CI: 3-34%). Although the response rate was moderate, the response time was promising.

In 18 patients with MCRPC in whom PDL-1 inhibitor avelumab was evaluated, no objective response could be obtained (12). However, in a small subgroup of five patients who received enzalutamide therapy with elevated PSA, three patients had stable disease lasting more than 24 months.

Clinical studies evaluating checkpoint blockers in prostate cancer have suggested that the use of these agents alone will result in less improvement than optimal in OS. However, these studies provide a perspective in terms of efficiency and should not be completely abandoned in this population. By combining with vaccines, hormonal agents or other modalities, further studies will help to understand the optimal approach to the use of checkpoint inhibitors in antitumor activity.

Therapeutic Cancer Vaccines

Sipuleucel-T showed improvement in OS in asymptomatic or minimally symptomatic MCRPC (13,14). Ultimately, it was the first cancer-approved therapeutic cancer vaccine of the FDA. These studies that change the practice have shown that prostate cancer is susceptible to immunotherapy and vaccination treatment is an effective and safe approach.

DCVAC/PCa

DCAVAC/PCa is an autologous vaccine and contains activated dendritic cells stimulated with killed PSA-positive LNCaP cells. The combination of DCVAC/PCa with standard dose docetaxel and prednisone was evaluated in 25 patients with MCRPC in a phase I/II, open label, single-arm clinical trial (15). The primary and secondary outcomes of the study were identified as safety and immune responses. The most common side effects were fatigue, back pain and paresthesia (all of them were gr1 or 2). As part of the safety assessment, OS was compared to the predicted values through the previously developed nomograms. OS with DCVAC/PCa regimen was 19 months and this result was reported to be significantly longer than the 11.8 months and 13 months predicted in the Halabi and MSKCC nomograms (HR: 0.26, 95% CI: 0.13-0.51).

The phase III study, VIABLE, is currently under way to further explore the potential of this promising treatment. VIABLE study was a randomized, double-blind, placebo-controlled, parallel group study and examined the efficacy and safety of placebo in 1200 patients with docetaxel + DCVAC/PCa versus docetaxel + placebo. The primary outcome is OS and the estimated end date of the study is June 2018 (16).

PROSTVAC

PROSVAC is a poxviral-based vaccine that encodes three co-stimulatory molecules (B7.1, ICAM-1 and LFA-3) together with PSA as the target antigen. In the phase II randomized, double-blind study with 125 patients with MCRPC, the PROSTVAC prime-boost regimen showed significant improvement in OS (17,18). The median OS with PROSTVAC was found to be 25.1 months and 16.6 months in the control arm (HR: 0.56, 95% CI: 0.37-0.85; p=0.0061).

In another study, the immune effect induced by PROSTVAC administration in 104 patients was evaluated (19). T-cell responses were compared before and four weeks after vaccination. Overall, 59/104 patients (57%) showed an increase in PSA-specific T-cell response, and 19/28 (68%) patients were shown to develop immune responses to tumor-associated antigens that were not present in the patient, and this concept is known as antigen spread.

These promising results paved the way for phase III study called PROSPECT (20). PROSPECT study is a double-blind study in asymptomatic or minimally symptomatic 1297 MCRPC patients, and patients were randomized to the PROSTVAC, PROSTVAC + GM-CSF or placebo arms. In the interim evaluation

conducted in September 2017, the primary outcome, OS, could not be reached and the study was terminated (21).

Although the results are disappointing, prospects for immunotherapy in prostate cancer may lie beneath the combination strategies. Studies on the combination of PROSTVAC with other immunotherapeutic agents or early cure of the disease continue.

GVAX-PCa

GVAX-PCa vaccine consists of cells derived from LNCap and PC3 cell lines and genetically modified to secrete GM-CSF. In a phase I/II dose escalation study performed on 80 patients with MCRPC, the vaccine was shown to be well tolerated and the most common side effect was erythema at the injection site (22). A significant proportion of 89% of the high dose group (p=0.002) has been reported to have an antibody against one or two cell lines.

Two phase III studies evaluating safety and OS were completed. In the first study, docetaxel + prednisone was compared to GVAX in MCRPC patients without chemotherapy (23). The study was terminated early because it did not meet the primary outcome, OS. The median survival in 626 patients analyzed was 20.7 months in the GVAX arm and 21.7 months in the control arm (HR: 1.03, 95% CI: 0.83-1.28; p=0.78). Grade III and above side effects were reported in 8.8% of the GVAX arm and in 43% of the docetaxel arm, and researchers reported that GVAX had a better toxicity profile. On the other hand, phase III study comparing GVAX + docetaxel with docetaxel alone in 408 MCRPC patients was terminated early due to imbalance in patient deaths (67 in vaccine group and 47 in docetaxel alone) (24). The imbalance was also reflected to the OS, and it was 12.2 months in the vaccine arm and 14.1 months in the chemotherapy arm (p=0.0076). Further analysis is required on the subject.

CV9104

CV9103 is a MRNA vaccine that encodes PSA, PSCA, PSMA and STEAP1 antigens (25). In a phase I/IIa study, 26 of 33 patients developed immune responses (25). OS was found to be significantly longer in patients who developed an immune response to multiple antigens than patients with no response or response to only one antigen (HR: 0.41, 95% CI: 0.17-0.95, p=0.017).

Second generation CV9103 formulations also encode PAP and MUC1 antigens in addition to the former (26). In phase IIb study, the improvement in primary outcome, OS, was not met in patients with asymptomatic or minimal symptomatic MCRPC (27).

Combination Strategies

The results regarding the effectiveness of the checkpoint inhibitors alone in the MCRPC have been disappointing so far. The mechanism behind this resistance must be clarified. Recent studies suggest that the tumor mutation load is predictive of a good response to PD1/PDL-1 (28,29,30) and CTLA-4 inhibitors (31). Prostate cancer is known to have a low mutation load (32). For this reason, it would seem that further evidence would support this hypothesis. Interestingly, in one study, it was concluded that the results obtained following a PD-1/PDL-1 inhibitor with CTLA-4 inhibitor were independent of the mutation load (28).

In a strategy, nivolumab with ipilimumab was evaluated in patients with advanced prostate cancer with androgen receptor mutation (33). As expected, a reduction of 50% PSA was achieved in one of 15 patients with accompanying toxicity, and in three out of 15 patients, persistent PFS was achieved (33).

Vaccines and Checkpoint Inhibitors

It is accepted that tumors with high PDL-1 expression in the tumor microenvironment tend to respond better to PD-1/PDL-1 inhibitors (34). In a study by Rekoske et al., it was shown that PDL-1 expression was increased in circulating tumor cells following the PAP-encoding DNA vaccine, and it was thought that there was a relationship between PDL-1 up-regulation and PFS (35). The researchers also found a trend with the sipuleucel-T vaccine targeting PAP.

In the phase I study consisting of 30 MCRPC patients and evaluating the safety and tolerability of ipilimumab and PROSTVAC, the most reported side effects were injection site reaction, colitis, rash, elevation in aminotransferases and endocrine side effects (36). The median OS was 34.4 months for all patients and two-year OS was reported as 73%. These results were found to be better than the previous vaccine alone and sipuleucel-T phase III studies. In particular, evidence has been obtained from this study for the spread of antigen by the immune response generated against tumor-associated antigens that are not present in the vaccine. Antigen spread may allow a more permanent and adaptive immune response that leads to improvement in long-term clinical outcomes (37).

In sipuleucel-T + ipilimumab study in nine patients, it was found that combination was well tolerated, and that postsipuleucel-T IgG and IgG-IgM levels were increased for PAP ($p<0.001$ and $p<0.0001$, respectively) and PA2024 ($p=0.0001$ and $p<0.000$, respectively) compared to baseline levels. Furthermore, it was reported that IgG and IgG-IgM levels for PAP ($p<0.001$ and $p=0.002$, respectively) and PA2024 ($p<0.0001$ and $p=0.001$, respectively) increased from postsipuleucel-T to postipilimumab. OS, spuleucel-T and PA2024 and PAP-specific immune responses were previously evaluated and considered to have the potential for clinical benefit of the checkpoint vaccine regimen (38).

Immunotherapy and Enzalutamide

Enzalutamide competitively inhibits androgen binding, nuclear translocation of the androgen receptor and its interaction with DNA. Immunological characteristics of this second generation antiandrogen, which has the advantage of survival in MCRPC, have been characterized (39,40). TRAMP mice were exposed to enzalutamide alone or in combination with the therapeutic vaccine by Ardiani et al. (41) and they reported increased thymic T-cell production and OS improvement in combination therapy compared to other therapies.

In the Phase II STRIDE study, 52 patients with MCRPC were randomized to either the concurrent or subsequent enzalutamide plus sipuleucel-T arms (42). According to the

results of intermediate immuno-analysis, the PA2024-specific T cell response was increased in both arms ($p<0.001$) (43). In both arms, cytokines such as INF-gamma, TNF-alpha and IL-2 were increased. There was no difference in toxicity in concurrent and subsequent applications.

Bishop et al. (44) have shown that patients with progression under enzalutamide treatment have more PD-L1/2 positive dendritic cells than patients who respond to enzalutamide or who are enzalutamide-naive.

When pemrolizumab was administered to patients who were under enzalutamide treatment, more than 50% PSA reduction was achieved in for out of 20 patients (45).

Immunotherapy and Abiraterone

Preclinical evidence suggests that abiraterone is also immunomodulatory, like enzalutamide (46). In a phase II study in 69 MCRCP patients comparing concurrent or subsequent abiraterone + prednisone and sipuleucel-T, the primary outcome was defined as cumulative antigen-presenting cell activation, and it has been shown that *ex-vivo* antigen-presenting cell activation and peripheral immune response increased in both arms compared to baseline ($p<0.05$) (47). This study also showed that low-dose prednisone did not affect the immunogenicity of sipuleucel-T.

In the phase I/II study where the primary outcome was safety, the combination of abiraterone + prednisone with ipilimumab was evaluated (48). The study was terminated due to toxicities such as grade 3 hypokalemia, dehydration and transaminase elevation.

Immunotherapy and PARP Inhibition

Olaparib is a PARP inhibitor and shows clinical activity in patients with MCRPC and DNA repair defect (49). Mutations in DNA repair genes such as *BRCA1*/2, ataxia-telangiectasia, Fanconi anemiagenes, and CHEK2 are observed in 1/3 of the patients. In patients with treatment-resistant MCRPC, the effect of olaparib and durvalumab is evaluated in the ongoing single-arm pilot study (50). In the intermediate analysis, it was reported that the combination had an acceptable toxicity profile in 10 patients and a PSA decrease of more than 50% was observed in 5/7 (71%) of the patients. Although the patient population has been less so far, the results of this study are particularly interesting given that they are given in an unselected population.

Conclusion

In large-scale studies other than sipuleucel-T, single-agent immunotherapies have not been shown to provide significant PFS and OS benefits in patients with MCRPC. The results of multiple phase III studies including ipilimumab and PROSTVAC were disappointing and revealed evidence that prostate cancer was not immunosensitive. However, benefit has been shown in smaller studies and it is thought that it is too early to abandon these agents completely. Determination of tumor and patient characteristics may be effective in response to immunotherapy. Combination strategies can overcome the escape from the immune response. The literature on avoiding the use of single-agent immunotherapy in MCRPC is increasing. Instead, resources should be concentrated on optimal patient selection and effective combinations to increase the immune response. Because therapeutic vaccines have a relatively low side-effect profile, research into their use in localized prostate cancer may be more valuable. Prostate cancer, showing biochemical recurrence, may be the optimal target population for immunotherapy regimens due to better toxicity profiles. Although checkpoint inhibitors are better tolerated than cytotoxic chemotherapies (51), these agents are associated with severe immune-mediated side effects. PD1/PDL-1 inhibitors are better tolerated than CTLA-4 inhibitors.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Review

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Targeted Agents and Resistance Mechanism in Renal Cell Cancer

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Abstract

Renal cell carcinoma (RCC) exhibits multidrug resistance protein P-glycoprotein expression due to the proximal tubular origin and in this regard, it is resistant to a large number of cytotoxic chemotherapy. The identification of the molecular pathogenesis, genetics, and epigenetics of RCC has led to new target points such as vascular endothelial growth factor. Tyrosine kinase inhibitors have been used mainly for treatment, but recently, immune checkpoint inhibitors have also been used in the treatment of RCC. Despite these treatments, response rates are not sufficient in the majority of patients. Primary resistance or acquired resistance to the treatment might be seen. Defining these resistance mechanisms will contribute to the management of the treatment and will help in to identify new treatment targets. In this review, we focus on the molecular mechanisms and resistance mechanisms of targeted-therapy.

Keywords: Renal cell carcinoma, resistance mechanisms, tyrosine kinase inhibitors,

Introduction

Renal cell carcinoma (RCC) emerges from renal tubular epithelial cells. Among newly diagnosed cancers, it is the 6th most common cancer in men (5%) and 10th in women (3%) (1). The most common subtypes are clear cell RCC (ccRCC) (75%), papillary RCC (10%) and chromophobe RCC (5%) (2). ccRCCs are the most common cause of RCC-related mortality (3). In the treatment of metastatic RCC (mRCC), targeted therapies have significantly improved the management of the disease in the last in last ten years. Agents that target vascular endothelial growth factor (VEGF) and mammalian target of Rapamycin (mTOR) pathways have proven to be effective in the treatment of mRCC, mainly in ccRCC. Recent immunotherapy studies with planned death-1 (PD-1) receptors and ligands (PD-L1) and "immune control point" targeted inhibitors such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) have changed the current practice.

In order to increase the efficacy of targeted therapies and to obtain more pronounced survival results, the molecular pathogenesis of RCC cases should be known in more detail and the prognosis of patients should be determined according to molecular characteristics. Through molecular biology research, RCCs have been identified at genetic and epigenetic levels, from single nucleotide polymorphisms to large chromosome defects (4).

Discussion

Molecular Pathogenesis of Renal Cell Carcinoma

In ccRCCs, von Hippel-Lindau (*VHL*) tumor suppressor gene loss is observed and changes in the genes involved in the chromatin remodeling complex are detected. Several studies have shown the association of ccRCC to lysine (K)-specific demethylase (KDM6A, *KDM5C*), histone methyltransferase SETD2 (suppressor of variegation, Enhancer of zeste, Trithorax-domain containing 2) and polybromo1 (*PBRM1*) genes in the chromatin remodeling complex (5).

The *VHL* tumor suppressor gene (*TSG*) is one of the earliest identified genes in the 3p25 locus related to ccRCC, and is present in 64-100% of ccRCC tumors as a driver mutation. The leading cause of death in 75% of patients with *VHL* syndrome is ccRCC. *VHL* protein (pVHL) binds hypoxia-induced factor α (HIFα) and induces ubiquitin-mediated proteolysis (6). Inactivation of the VHL gene formed by mutation, deletion or methylation leads to the accumulation of HIFα under normal oxygen conditions without hypoxia, and promotes tumor growth. Genetic changes in the VHL gene are believed to be

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related only to ccRCC, however, van Houwelingen et al. (7) identified VHL gene mutations in 15% of non-ccRCCs in their study on a group of sporadic RCC patients in the Netherlands. However, in other studies, no mutation in the VHL gene was observed in other RCC subtypes (8,9).

The discovery of the VHL gene in familial and sporadic ccRCC has revolutionized the treatment of advanced RCC. Treatments aimed at suppressing angiogenesis by VEGF or platelet-derived growth factor (PDGF) mediated pathways have replaced immunotherapies such as interferon alpha (IFNα) and interleukin-2 (IL-2), which are used in the treatment of mRCC. Tyrosine kinase inhibitors (TKIs) (axitinib, cabozantinib, lenvatinib, pazopanib, sorafenib and sunitinib), VEGF monoclonal antibody (bevacizumab) and mTOR inhibitors (everolimus and temsirolimus) have received FDA approval as targeted treatment drugs for RCC (10).

In ccRCC, the VHL gene is not the only genetic deviation in the chromosome 3p region. After the VHL gene, the mutation in the gene of Fragile histidine triad protein (FHIT) found in the region 3p14.2 was observed in about 40% of the cases. The chromosomal translocation t (3;8) (p14.2; q24) was first identified in hereditary RCC and was then found in sporadic ccRCC as a widespread loss site in chromosome 3 (11). The specific function of the FHIT protein is still unclear. Another *TSG* associated with ccRCC in the 3p chromosomal region is the Ras association domain family 1 isoform A (*RASSF1A*) gene found in 3p21.3. *RASSF1A* protein regulates microtubule formation, cell cycle control and apoptosis (12). The *RASSF1A* promoter region becomes inactive by hypermethylation and is frequently seen in ovarian, breast and lung cancers besides ccRCC (13). *RASSF1A* inactivation is present in approximately 44% of papillary RCCs (14). Recently, next-generation sequencing or exon sequencing studies have shown several new genes related to chromatin modification in ccRCCs (15). Newly defined genes are *PBRM1*, AT-rich interaction domain 1A (*ARID1A*), *BRCA1* associated protein-1 (*BAP1*), *SETD2*, and lysine-specific demethylase 5C (*KDM5C*) (16). *PBRM1* mutations were detected in 41% of ccRCCs. All *PBRM1*, *BAP1* and *SETD2* genes are described to be inactivated in a similar manner to VHL near-3p21 region by Knudson's two-hit hypothesis (17).

DNA sequence analysis, transcriptome and integrated data analysis in recent studies have revealed frequently mutated signaling pathways such as phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR and p53 in ccRCC (18,19). PI3K-AKT-mTOR pathway regulating angiogenesis, cell cycle progression and proliferation is the target of mTOR inhibitors (temsirolimus and everolimus). mTOR, phosphatase and tensin analog (PTEN), *PIK3CA*, AKT2 and other genes in this pathway have been shown to be mutated in 26-28% of ccRCC tumors.

Molecular Pathways in Targeted Treatment of Renal Cell Carcinoma

After understanding the molecular pathogenesis of RCCs, targeted therapies have been developed. In the treatment of RCC, inhibition of signaling pathways that play an important role in carcinogenesis is targeted. Research has shown that angiogenesis is excessive in RCC. Due to these results, the inhibition of the angiogenesis pathway is prominent in targeted therapies. The most important gene associated with angiogenesis in RCC is the VHL gene. This has led to the use of agents that primarily target the VHL/HIF pathway in the treatment of RCC.

Anti-angiogenic agents targeting VEGF and mTOR pathways have proven their efficacy in the treatment of mRCC. Recently, immunotherapy with CTLA-4 and PD-1 receptors and "checkpoint" targeted inhibitors such as PD-L1 has been included in the treatment of RCC. Numerous clinical studies, particularly combination therapies, continue.

VEGF Inhibitors

VEGF-tyrosine kinase receptor inhibitors (axitinib, pazopanib, sorafenib, sunitinib) block the signaling pathway by binding to the intracytoplasmic region of the receptor. Pazopanib is an angiogenesis inhibitor that acts by VEGF receptor (VEGFR) -1, -2 and -3, and PDGF receptor (PDGFR) α, β and c-kit. In phase III study, pazopanib was compared with placebo, and the progression-free survival (PFS) was longer (9.2 months vs. 4.2 months) and the objective response rate (ORR) was higher (30% vs. 3%) in the pazopanib group (20). In the updated results of survival and safety analysis, the reason why pazopanib treatment did not show a survival advantage was attributed to the permission to pass from placebo group to pazopanib group after disease progression (21).

Sunitinib is a multi-target tyrosine kinase inhibitor acting via VEGFR-1, -2, and -3, PDGF-Rα and β, c-kit, FLT-3, colony stimulating factor receptor (CSF-1R), and neurotrophic factor receptor (RET). In phase III study, sunitinib and IFNα were compared in the first-line treatment. The PFS was longer (11 months vs. 6 months, respectively) and ORR was higher (34% vs. 6%, respectively) with sunitinib treatment. Sunitinib was approved by the FDA for the first-line treatment of mRCC (22).

Both agents showed similar efficacy in phase III COMPARZ study, a non-inferiority study comparing sunitinib with pazopanib (23,24).

Bevacizumab is a monoclonal antibody that binds to circulating VEGF A with high affinity and inhibits signal transduction. In the phase III AVOREN study, the combination of IFNα and bevacizumab was compared with the combination of IFNα and placebo, and PFS in the bevacizumab group was approximately five months longer (10.2 months vs. 5.4 months) (25).

Sorafenib, similar to sunitinib, have inhibitory effects on VEGF, PDGF-R, c-kit and c-MET. In the comparative study, the median PFS was 5.5 months with sorafenib and 2.8 months in the placebo group. Overall survival (OS) was 17.8 months in the sorafenib group and 14.3 months in the placebo group (p=0.0287) (26).

Different current treatment options and their rankings in mRCC treatment are summarized in Table 1 and Table 2.

In the AXIS study comparing axitinib with sorafenib in the second line treatment of mRCC, higher ORR and longer PFS were shown in the axitinib group (27). Afterwards, the efficacy of axitinib in the first line treatment was investigated. In a study comparing axitinib treatment with sorafenib in the first line treatment, PFS was 10.1 months in the axitinib group and 6.5 months in the sorafenib group (28).

Cabozantinib is a newly developed, powerful VEGF-2 and c-MET dual inhibitor. Recent studies have shown that MET signals are important in sustaining VEGF signals, tumor angiogenesis, proliferation and patient survival (29). These results also indicate that MET signaling may play a role in VEGF inhibitor resistance. In the METEOR study comparing cabozantinib with everolimus in previously treated patients, cabozantinib has been shown to contribute to PFS for approximately four months (7.4 months vs. 3.8 months) (30) and to OS for approximately five months (21.4 months vs. 16.5 months) (31). Dovitinib is a new TKI that targets both VEGF and fibroblast growth factor receptor (FGFR) pathways. Preclinical studies have also shown that this dual-acting TKI has activity against topoisomerase (32). In the phase III study, median PFS with dovitinib and sorafenib treatments were found to be 3.7 and 3.6 months, respectively, and dovitinib did not show an additional contribution to clinical benefit compared to sorafenib treatment. In a phase II study comparing lenvatinib, the combination of lenvatinib and everolimus and everolimus alone, the combination therapy showed PFS and OS advantage against everolimus alone (PFS: 14.6 months vs 5.5 months; OS: 25.5 months vs 15.4 months) (33,34) and was included in the post-first line treatment sequence.

Table 1. mRCC treatment options (clear cell carcinoma dominant)

	First-line	Second-line
Tyrosine kinase inhibitor	Axitinib Cabozantinib Pazopanib Sunitinib	Axitinib Cabozantinib Lenvatinib + everolimus Pazopanib Sorafenib Sunitinib
VEGF receptor antibody	Bevacizumab + IFNα2b	Bevacizumab
mTOR inhibitor	Temsirolimus	Everolimus Temsirolimus
Immunotherapy	HD-IL2 Nivolumab	Nivolumab HD-IL2

VEGF: Vascular endothelial growth factor, mTOR: Mammalian target of rapamycin, HD-IL2: High-dose interleukin-2, IFNα2b: Interferon alpha and interleukin-2, MRCC: Metostatic renal cell carcinoma

Table 2. mRCC treatment options (sub-types except for clear cell carcinoma)

Tyrosine Kinase Inhibitor	Axitinib Cabozantinib Lenvatinib + everolimus Pazopanib Sorafenib Sunitinib
VEGF receptor antibody	Bevacizumab Bevacizumab + erlotinib Bevacizumab + everolimus
mTOR inhibitor	Everolimus Temsirolimus
Immunotherapy	Nivolumab

mRCC: Metastatic renal cell carcinoma, VEGF: Vascular endothelial growth factor, mTOR: Mammalian target of rapamycin

mTOR Inhibitors

The second important treatment target is the mTOR pathway. The *PI3K*/AKT/mTOR pathway plays an important role in many cancers (35). The binding of VEGF to the VEGF receptor causes activation of the mTOR pathway. Activation of this pathway provides cell growth, proliferation, angiogenesis, mobility and survival function, as well as activating protein synthesis and transcription in the cell. Active mTOR pathway accelerates the translation of both ribosomal protein and tumor progression factors, as well as activation of HIF and cell cycle regulators. In addition, the *PI3K*/AKT/mTOR pathway was modified in 28% of tumors (36). Blocking the mTOR signal leads to a reduction of protein translation, angiogenesis and inhibition of tumor cell proliferation. The mTOR protein consists of mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) found in the cytoplasm. This complex acts as a regulator in processes such as cell metabolism, growth and proliferation. In targeted therapies, mTOR inhibitors inhibit mTORC1 only. Drug resistance resulting from treatment with temsirolimus or everolimus develops as a result of mTORC2 activation in the background (37).

Temsirolimus blocks mTORC1 by forming a complex with FK-506 binding protein (FKBP) in mRCC (37).

In phase III ARCC study comparing temsirolimus with IFN therapy in first-line treatment in patients with poor prognosis in mRCC, temsirolimus treatment has been shown to statistically significantly contribute to survival (10.9 months in temsirolimus and 7.3 months in IFN groups) (38).

Comparing sorafenib and temsirolimus as second-line treatment after sunitinib, the benefit of temsirolimus treatment in improving PFS or OS has not been shown (39).

Everolimus is a mTOR inhibitor used in second- or third-line therapy in mRCC that progresses after VEGF-TKI treatment (40).

Immunotherapy

Prior to 2005, cytokines (INFα and IL2) were the most effective treatment agents used alone or in combination for the treatment of mRCC. High-dose interleukin-2 (HD-IL2) was the only treatment option for long-term response to mRCC treatment. Long-term responses can be obtained with HD-IL2 in 5-7% of patients. These therapies have given way to less toxic and more effective TKI treatments. Combination of IFNα with bevacizumab is among the recommended treatment options for mRCC.

CTLA-4 inhibitors (Ipilimumab) and PD-1/PD-L1 inhibitors (Nivolumab) have been used in clinical practice in the treatment of mRCC (41).

In phase III CheckMate 025 study comparing nivolumab with everolimus in second-line treatment of mRCC, nivolumab was shown to contribute approximately 5.4 months to survival (25 months vs. 19.6 months) (42) and it has taken its place in the second line treatment. In the CheckMate 214 study, the combination of nivolumab and ipilimumab was compared with sunitinib in the first-line treatment. The median overall survival could not be achieved with nivolumab and ipilimumab combination especially in patients in the middle and poor risk groups, and median survival was 26 months in the sunitinib

group. However, for the patients in the good risk group, sunitinib clearly maintains its place in the first step (43).

PD-1 is a member of the CD 28 receptor family and they are expressed in naive and activated T, B-lymphocytes and natural killer (NK) cells in peripheral blood. PD-1 has two ligands, namely PD-L1 and PD-L2. When PD-1 binds to its ligand during recognition, it causes PD-1 to cross-link with the antigen receptor complex. Phosphorylation of the PD-1 receptor then leads to the recognition of SHP2, and inactivation of ZAP 70 in T cells and Syk in B cells (44). PD-1 activation induces inhibition of cell growth and cytokine secretion. It has also proven to be an important regulator in the immune response process and in the tolerance to environmental immunity. In tumor cells, it has been shown that PD-1 expression level increases, cytokine production is decreased and cytotoxicity of the tumor is impaired. Blockage of PD-1/PD-L1 pathway showed effective anti-tumor activity in Phase I/II studies (45,46).

In Phase II IMmotion150 study comparing sunitinib with combination of atezolizumab and bevacizumab, PFS was found to be 7.8 months vs. 14.7 months, respectively, in mRCC patients with PD-L1 staining more than 1% (47).

Resistance Mechanisms Against Targeted Treatments in Renal Cell Carcinoma

Studies have shown that there are two types of resistance to TKI treatment, including primary resistance and acquired resistance. Resistance to TKI treatment can be overcome by second-line treatments (mTOR, c-MET, PD-1 inhibitors or a combination of a mTOR inhibitor with a TKI).

Primary Resistance

The primary or intrinsic resistance mechanism is determined depending on the molecular properties of each tumor. Targeted therapies (VEGF-targeted inhibitors) have no clinical benefit for patients with this type of resistance. Gordan et al. (48) identified three groups of ccRCCs that were based on HIF-α detection and could explain intrinsic resistance: patients with detected HIF-α protein and wild-type VHL alleles, patients with VHL defective tumors (24% methylation, 4% homozygous deletion) and detected HIF-1α and HIF-2α expression, and patients with VHL defective tumors expressing HIF-2α only (24% methylation). These findings suggest that HIF-1α and HIF-2α promote different oncogene activation in ccRCC. The other mechanism presumed to cause primary resistance is the presence of pro-angiogenic signals that bypass the TKI inhibition and thus allow angiogenesis to continue (49). Other mechanisms associated with primary resistance include increased expression of B cell lymphoma-2 (Bcl-2) and/or Bcl-XL proteins that are important in inhibition of apoptosis and a reduction in expression of CD95 (50).

Epigenetic modifications of histone protein in chromatin have been shown to play an important role in the regulation of gene transcription patterns in cells by the catalytic activity of histone deacetylase and methyltransferase. Changes in genes encoding these enzymes have been identified in RCC (36). In recent studies, it has been shown that inactivation of antiangiogenic factors by methylation of the histone methyltransferase at the promoter site of EZH2 contributes to tumor angiogenesis. EZH2 overexpression contributes to the development of resistance to TKI treatment (51).

Conclusion

Acquired Resistance

Resistance to TKI treatments may be possible with various mechanisms. As a result of various studies, it has been shown that the regulation of angiogenesis-related genes around the tumor, increasing the rate of pericyte cells in the vascular bed, removal of pro-angiogenic inflammatory cells from the bone marrow, or increasing the ability of tumor cells to invade healthy tissues are effective in acquired resistance in resistant RCCs (49). Despite the various mechanisms described for resistance to targeted-therapy, currently there are no biomarkers available to describe drug resistance in patients.

Activation of Alternative Pro-angiogenic Pathways

Activation of alternative pro-angiogenic pathways independent of VEGF after TKI treatment is one of the most common resistance mechanisms. The studies showed overexpression of pro-angiogenic factors such as fibroblast growth factor 1 and 2 (FGF1/2), IL-8, efrin A1 and A2 (Efna1/2) and angiopoietin 1 and 2 (Ang1/2) as a result of hypoxia induced by anti-angiogenic therapy (52). FGF can directly stimulate endothelial cell proliferation and the formation of endothelial tubules in the presence of TKI. IL-8 is a pro-angiogenic factor with high expression in TKI-resistant patients, and its expression is regulated by the transcription factor NF-κB independent of the HIF-1α pathway.

IL-8 binds to the CXCR2 receptor and leads to autocrine activation of VEGFR-2 and consequently increased angiogenesis as a result of proliferation of VEGF mRNA and its dependent protein expression in endothelial cells (53). Ang 2 expression significantly increases with hypoxia and loss of VHL gene in RCC. Ang 2 functions as a natural antagonist of Ang 1 and is only expressed in active angiogenesis, vascular remodeling, pathological angiogenesis processes in tumors (54). However, the function of Ang 2 may vary depending on other pro angiogenic signals.

Placental growth factor (PlGF) is a VEGF homolog which is expressed by tumor cells, endothelial cells, bone marrow-induced pro-angiogenic cells, inflammation cells, and stromal cells, and which binds to VEGFR-1. Binding of PlGF to VEGFR-1 stimulates angiogenesis. The PlGFs/VEGFR-1 complex strengthens the VEGFR-2 signals and thus increases angiogenesis. In addition, PlGF enhances the expression of VEGF-A, FGF2, PDGFβ and matrix metalloproteinases (MMPs) and stimulates angiogenesis through various mechanisms (54).

VEGF-targeted therapy may cause metabolic stress in cancer cells that are exposed to oxygen and nutrient deficiency as a result of angiogenesis inhibition. In response to this, cells activate alternative signaling pathways such as the *PI3K*/AKT/mTOR pathway for amino acids and other energy sources required for protein synthesis, cell growth and proliferation. It has been suggested that activation of *PI3K*/AKT/mTOR pathway

correlates with aggressive RCC tumor behavior and poor prognosis (55,56).

Resistance Induced by Tumor Microenvironment

Tumor stroma is composed of endothelial cells, fibroblasts, pericytes and hematopoietic cells. These cells play an important role in angiogenesis and tumorigenesis by directly contributing to vascularization or secreting angiogenic factors (VEGF and MMP-9). Tumor-associated fibroblasts play an active role in tumorigenesis and may develop resistance to antiangiogenic therapy with VEGF inhibitors, and increased PDGF-C expression plays a role in this resistance mechanism (57). Pericytes are another type of stromal cell that directly contributes to the formation of blood vessels. The binding of PDGF-BB in endothelial cells to PDGFR-β in pericytes leads to increased VEGF mRNA transcription by MAPK and *PI3K* in pericytes, and induces the survival of endothelial cells in a paracrine way (58).

Increasing pericyte cell count and VEGF production may result in increased survival of endothelial cells, and may make endothelial cells less susceptible to VEGF inhibition signals. On the other hand, the reduction of pericytes and loss of function cause the loss of vessel stabilization, resulting in vascularization of tumor cells and thus facilitating metastasis (59). Because of hypoxia resulting from the regression of tumor vessels, pro-angiogenic inflammation cells, such as CD11b + Gr1 + myeloid-derived suppressor cells (MDSC), increase in the tumor microenvironment (60). These cells can also be added to tumor endothelium and differentiate into endothelial cells. Angiogenesis is activated independently of VEGF in the tumor as a result of a higher expression of the pro-angiogenic Bombina variegata factor in CD11b + Gr1 + MDSC in resistant tumors.

Increased Invasive Feature and Metastasis

It has been suggested that the ability of tumors to metastasize to other cells increases with tumor hypoxia. Hypoxia also stimulates the expression of c-MET receptors. c-MET activation leads to tumor cell proliferation, increased survival and increased invasiveness with various signaling pathways such as *PI3K*/AKT, MAPK, Src and STAT3 (61). Recently, increased activity of c-MET has been shown to increase epithelial-mesenchymal transition (62). After a prolonged extracellular stimulation, the accumulation of protein in the cell leads to cellular changes and the epithelial cells are freed from their typical biological structures. The expression of molecules such as platelet endothelial cell adhesion molecule 1 (PECAM1/CD31), homeobox A9 and endothelial cell-specific molecule 1 is reduced in these cells (63). Expression of MMPs increases and thus leads to polarization and deformation by allowing cell-cell adhesion and decreased cell penetration (63). Reduction of cell-cell adhesion and increased expression of MMPs increase the invasion characteristics of cells to other tissues.

Lysosomal Sequestration

The preclinical study results showed that the concentration of sunitinib, known as intracellular TKI, was ten-fold higher in resistant cells than sensitive cells. The hydrophobic structure of sunitinib allows this molecule to easily pass through the lysosomal plasma membrane, but the acidic environment of the lysosome does not allow its release. These results support the idea that sunitinib is retained in lysosomes. This mechanism protects the cell against antiangiogenic activity despite its high intracellular concentration of sunitinib. This mechanism provides a new model for transient acquired resistance (64). Lysosomal sequestration as a resistance mechanism has been proven to be reversible (65).

Single-nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) found in genes that regulate pharmacokinetics and pharmacodynamics of TKIs may play a role in the development of resistance to VEGF-targeted therapy (66). SNPs in NR1I2 and NR1I3 nuclear receptor genes may negatively affect PFS and/or OS by negatively regulating CYP3A4 expression. Single nucleotide polymorphisms with pharmacodynamic factors such as TKI targets (VEGFR and PDGFR) may also contribute to the development of resistance to TKI (67).

Resistance Through MicroRNAs

In microRNA (miRNA) profile studies, different miRNA patterns were identified in RCC. The expression of miRNA-942, miRNA-133a, miRNA-628-5p and miRNA-484 was higher in TKI-resistant RCC tumors compared to TKI-sensitive tumors. Overexpression of miRNA-942 in an RCC cell line increased MMP-9 and VEGF release and caused the migration of endothelial cells and increased treatment resistance (68).

Prevention of Resistance to Treatment in RCC

There are different strategies to prevent and overcome resistance to TKIs in the treatment of RCC. These include transition to a different alternative drug (either a VEGF or mTOR targeted therapy) and combined therapies (69). The underlying mechanisms for poor results in patients with primary resistance are complex. Understanding and preventing these mechanisms is important. As a result of preclinical studies in this direction, the efficacy of trametinib, a MEK inhibitor drug developed against RAS/MEK/ERK and *PI3K*/AKT pathways, has not yet been demonstrated in metastatic RCC patients (70).

Replacement with the Same Drug Group

Treatment with a TKI and a different subsequent TKI gave positive results in patients with advanced RCC. This approach is based on the fact that different TKIs have different target profiles and potential (71). Sunitinib targets multiple kinase receptors including VEGFR-1, 2 and 3, PDGFR-α and β, c-KIT, FLT-3, CSF-1R and RET. On the other hand, sorafenib inhibits targets found in tumor cell (CRAF, BRAF, V600E BRAF, c-KIT and FLT-3) and tumor vasculature (CRAF, VEGFR-2 and 3 and PDGFR-β) (72). In patients with metastatic RCC, the anti-tumor activity of axitinib has been shown after treatment failure with sorafenib and sunitinib (71).

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Consept: Y.Ü., Design: S.R., Y.,Ü., Data Collection or Processing: S.R., H.A.Y., Analysis and/or Interpretation: S.R., Y.Ü., Literature Search: S.R., H.A.Y., Writing: S.R., H.A.Y., Ç.A.

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Review

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Nuclear Medicine Applications in Diagnosis of Urological Tumors

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Abstract

Except for prostate carcinoma, there is limited data in the literature on the role of nuclear imaging methods in the management of urological cancers. 18F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) is generally the most widely used method in oncological imaging. However, the diagnostic power of this radiopharmaceutical in urological tumors is weakened partially due to its physiological urinary excretion. For this reason, some other 18F-labeled molecules, especially Ga-68 prostate-specific membrane antigen for prostate cancer and 18F-sodium fluoride for bone metastases, have recently gained importance. In addition, characterization of renal masses with Tc-99m methoxy isobutyl isonitrile (MIBI), a nonspecific tumor agent, and detection of bone metastases with whole-body Tc-99m methylene diphosphonate (MDP) bone scintigraphy are still used. In this review, scintigraphic methods and PET/CT imaging used in diagnosis and follow-up of urological tumors will be discussed.

Keywords: Urological neoplasms, radionuclide imaging, radiology

Introduction

Computerized tomography (CT) is the most commonly used imaging method for diagnosis, staging, treatment planning and follow-up of kidney tumors and bladder tumors. Magnetic resonance imaging (MRI) can provide more detailed information about local advanced disease. In particular, the use of multiparametric MRI in prostate cancer is increasing (1).

Nuclear imaging methods can provide information on the function, behavior and receptor status of tumoral tissue, unlike anatomical imaging. 18F-fluorodeoxyglucose (18F-FDG) is the most commonly used metabolic agent in oncologic PET/CT studies and provides whole-body evaluation in one step. Although urinary excretion of 18F-FDG in urological tumors is intense and thus its role in local disease evaluation is limited, it provides a significant advantage by demonstrating extent of disease. Imaging studies with new radiopharmaceuticals undergoing lower urinary excretion continue.

Kidney Tumors

18F-FDG PET/CT

Sensitivity of 18F-FDG PET/CT has been reported between 50% and 60% in the primary diagnosis and determination of kidney tumors. For this purpose, it has not been shown to have a significant contribution to conventional imaging methods such as CT and MRI. In the literature, although imaging after forced diuresis or dual-phase delayed imaging method have been tried in order to reduce the effect of physiological urine activity, no superiority was achieved with these methods over routine imaging protocol. In addition, it was found that there was no correlation between the amount of GLUT 1 expression and 18F-FDG uptake after surgical excision of the primary tumor (2,3,4).

Routine use of 18F-FDG PET/CT in the staging of kidney tumors is not recommended in standard protocols and guidelines. The most important reason for this is that 18F-FDG has a high rate of false negativity for the primary tumor due to the intense physiological urine activity. However, it has been reported that it may be useful in demonstrating extrarenal metastatic disease in risky patients (5,6).

Early detection and treatment of recurrence after nephrectomy shows a certain survival benefit for some patients. As a whole-body imaging method, 18F-FDG PET/CT can make a significant contribution to patient management during the restaging phase. Metabolic characterization can provide more accurate diagnosis in patients with recurrent or metastatic suspicious findings in postoperative follow-up radiological imaging results. In addition, it is more successful in detecting bone metastases than whole-body bone scintigraphy. The studies published in the literature are small sample studies and mostly retrospective. Recently, a meta-analysis of the results of 1158 patients in 15 studies was published and the sensitivity and the specificity of

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18F-FDG PET/CT were 86% and 88%, respectively (7,8,9,10). The sensitivity and specificity of 18F-FDG PET/CT in restaging in 104 patients with renal cell carcinoma (RCC) at postoperative 2nd year were 74% and 80%, respectively. In this series, follow-up treatment strategies were changed in 43% of patients with 18F-FDG PET/CT. In this study, patients with and without pathologic uptake in 18F-FDG PET/CT were compared in terms of survival, and three-year progression-free survival and five-year overall survival rates were significantly lower in patients with positive PET/CT compared to patients with normal PET/CT (20% vs 67% for progression-free survival, 19% vs 69% for overall survival). Thus, 18F-FDG PET/CT can also be used as a prognostic marker in the follow-up of patients with kidney tumors, besides its ability to detect recurrence or metastasis (11). However, routine use is not recommended with existing data and the results of prospective studies to be performed in large patient groups are needed in order to better determine its role in the staging (Table 1).

In RCC, partial/radical nephrectomy or local ablative treatments are performed as definitive treatment in the presence of local disease. In advanced stage disease, anti-angiogenic agents or immune checkpoint inhibitors targeting the vascular endothelial growth factor pathway are used alone or in combination. Since these treatments are very expensive and require close follow-up in terms of the side effects profile, it is important to determine the patients who will benefit from the treatment in the early period in order to prevent both complications and unnecessary treatment costs. As the response evaluation criteria in solid tumors (RECIST) criteria predict, only size-based assessment may not reflect the actual clinical response in this patient group, especially in patients with bone metastasis. A clinical response can be achieved and survival may be prolonged, even if the lesion size is very small or the lesion is growing. Therefore, other methods were searched for the evaluation of the actual treatment response and 18F-FDG, which is the most frequently used agent for evaluating the metabolic response, was tried. When the data of a few studies were examined, it was demonstrated that its role might be important in the evaluation of response in patients using tyrosine kinase inhibitors, that the change between baseline maximum standardized uptake value (SUV_{max}) values and post-treatment SUV_{max} values could be prognostically significant and that the prognosis was worse in patients with higher activity in baseline 18F-FDG PET/CT study (12,13,14,15,16,17,18,19,20,21).

18F Florotymidine (18F-FLT) PET/CT

Another PET agent, which is tried for restaging in the follow-up of RCC, is 18F-FLT. 18F-FLT is a proliferation agent that remains in the cell by phosphorylation with thymidine kinase in proliferating tumors. Since thymidine is not a substrate of phosphorylase, it undergoes glucuronidation and is kept intensely in the liver and bone marrow in the body (22). In a multicenter study comparing the role of 18F-FDG PET/CT with 18F-FLT PET/CT in evaluating the treatment response of patients treated with sunitinib for diagnosis of metastatic RCC, it has been reported that baseline 18F-FDT PET/CT has a prognostic value, and that 18F- FLT PET/CT does not have such a benefit but it can be used much earlier in the evaluation of response to treatment than in 18F-FDG (1-2 weeks) (23).

Ga-68 Prostate-specific membrane antigen PET/CT

Renal cell cancers are highly vascular tumors. A high (75-97%) expression of PSMA was shown in the neovascularization bed (24). Therefore, Ga-68 PSMA was also tested in the diagnosis and follow-up of RCC. Although it is not effective in demonstrating primary tumor due to renal excretion, it is an agent that can be useful in the characterization of lesions that are considered as suspicious by conventional methods (25). Higher uptake is observed in clear cell carcinoma than in papillary type (26,27). In the literature, the data on this subject consisted of case reports and case series, and the sensitivity and positive predictive value of Ga-68 Prostate-specific membrane antigen (PSMA) PET/CT were better compared to CT (92% vs 69% and 97% vs 80%) (28).

Tc-99m Myocardial Perfusion Imaging Test SPECT/CT

Benign and malignant differentiation cannot be performed by conventional methods in 14% of operated T1 kidney masses (<4 cm), and pathological results of 20-30% of operated cases are reported as benign. Thus, although no PET agent can be shown for preoperative characterization of primary renal masses, there is a SPECT agent that may be useful. Tc-99m MIBI is a nonspecific tumor agent used for imaging by conventional gamma cameras. In benign and malignant tumors with increased metabolic rate, it is retained in mitochondria within the cell (29). Because oncocytomas contain more mitochondria than other types of RCC, they show higher Tc-99m MIBI uptake (30). When the results of the few studies on this subject were evaluated, Tc-99m MIBI was positive in almost all of the

Table 1. Diagnostic value of 18F-FDG PET/CT in staging and re-staging of renal tumors

Authors	Number of Patients	P/R	Indication	Sensitivity (%)	Specifity (%)	Accuracy rate (%)
Kang D et al. (5)	66	R	S	Primary tumor: 60, RPLN: 75, Distant metastasis: 75-77.3	Primary tumor: 100 RPLN: 100 Distant metastasis: 97-100	-
Özülker et al. T (8)	18	P	S	Primary tumor: 46.6	Primary tumor: 66.6	50
de Llano et al. S (9)	58	R	RS	80.56	86.36	58.7
Kumar et al. (10)	63	R	RS	90	91	90
Alongi P et al. (11)	104	R	RS	74	80	-

P: Prospective, R: Retrospective, S: Staging, RS: Restaging, RPLN: Retroperitoneal lymph node

patients who were diagnosed as oncocytoma pathologically and who were evaluated with Tc-99m MIBI SPECT/CT in the preoperative period, and Tc-99m MIBI uptake was not observed in patients diagnosed as having other RCC subtypes. Sensitivity for oncocytomas was reported as 83-100% (31). In a recent study, Tc-99m MIBI SPECT/CT was performed in 48 patients who had T1 tumors before the nephrectomy, and Tc-99m MIBI SPECT/CT was positive in nine patients with pre-operative benign diagnosis. Out of these nine patients, pathology report was compatible with oncocytoma in seven patients and chromophobe RCC in two patients. Five patients with negative Tc-99m MIBI SPECT/CT were confirmed to have RCC in the postoperative period (32).

Bladder Tumors

18F-FDG PET/CT

Its role in the detection of primary bladder tumor is limited due to urinary excretion of radiopharmaceuticals, as in all urologic tumors. No superiority to CT or MRI was demonstrated (33). In lymph node staging, the sensitivity was reported as 46-82%, the specificity was 89-97%, and the accuracy rate was reported as 84-92%. It was reported that it contributed to the conventional imaging methods in 20-40% of the patients and caused a change in treatment management in 68% (34,35,36,37). In order to determine its role in restaging after primary treatment, large series are needed. In a study conducted in 35 patients, it was reported that 17% of the patients had a change in the planned treatment strategy after 18F-FDG PET/CT (38,39). There are publications showing that it can be useful than conventional methods in the differentiation of residual tumor and necrosis for evaluation of neoadjuvant chemotherapy response (40,41). It was reported that occult metastases which cannot be demonstrated by radiological imaging methods in patients with muscle invasive bladder tumor could be demonstrated by 18F-FDG PET/CT and that preoperative 18F-FDG PET/CT positive patients have worse survival compared to negative patients (median overall survival 14 vs 50 months, progression-free survival 16 vs 50 months, $p<0.001$). In addition, the presence of extravesical lesion was shown to be an independent prognostic marker by multiple variance analysis (42,43) (Table 2).

C-11 Choline PET/CT

C-11 choline is phosphorylated by choline kinase after being taken into the cell and incorporated into the structure of cell membrane phospholipids. C11-choline uptake was also increased in tumors with increased proliferation rates (44).

In the functional imaging of bladder tumors, agents with less urinary excretion than 18F-FDG were tested. Since C-11 choline is a radiopharmaceutical with short half-life, it is thought that the need for faster imaging after injection would minimize handicaps due to physiological urinary excretion. However, in a few studies, the sensitivity in demonstrating lymph node metastases before radical cystectomy was found to be low. For this purpose, its superiority to CT has not been proved. It may be more useful in patients with recurrence after cystectomy (Table 2) (45,46,47,48).

C-11 Acetate PET/CT

In the literature, the accuracy rates of CT and MRI and C-11 acetate PET/CT have been shown to be similar and it is stated that it is not superior to C-11 choline (49,50). In a recent study by Salminen et al. (51), C-11 acetate PET/MR has been reported to have high sensitivity and accuracy rates in detecting muscle invasive bladder cancer and response to neoadjuvant chemotherapy in these patients and to have limited success in lymph node staging (Table 2).

Prostate Cancer

Ga-68 PSMA PET/CT

Prostate-specific membrane antigen (PSMA) is an integral protein found in the neovascularized endothelial cell membrane, not in the tumor itself. In prostate cancer, it is 10 times more expressed than non-cancerous prostate. In the literature, there are studies conducted with more than one PSMA ligand labeled with Ga-68 and the most widely used is PSMA 11 (52,53,54).

Ga-68 PSMA uptake is known to increase in dedifferentiated, metastatic, hormone refractory disease. PSMA expression level is closely related to Gleason score, serum PSA level and prognosis (55,56,57). In demonstration of primary tumor in moderate-high-risk disease, the sensitivity and specificity of

Table 2. Diagnostic value of 18F-FDG, C-11 choline and C-11 acetate PET in staging and re-staging of bladder tumors

Authors	Number of Patients	P/R	Radio-pharmaceutical used	Indication	Sensitivity	Specifity (%)	Accuracy Rate (%)	Changes in clinical approach
Apolo AB et al. (34)	47	P	18F-FDG	S	87	88	-	-
Swinnen G et al. (36)	51	P	18F-FDG	S	46	97	84	-
Jadvar H et al. (38)	35	R	18F-FDG	RS	-	-	-	17
Kibel AS et al. (42)	43	P	18F-FDG	S	70	94	-	-
Drieskens O et al. (39)	40	P	18F-FDG	S	60	88	78	-
Gofrit et al. (46)	18	R	C-11 Choline	S	100	92	-	-
Brunocilla E et al. (47)	26	P	C-11 Choline	S	43	84	-	-
de Jong et al. (48)	18	R	C-11 Choline	S	67	100	-	-
Vargas et al. (49)	16	P	C-11 Acetate	S	100	71	-	-

P: Prospective, R: Retrospective, S: Staging, RS: Restaging

were 58% and 82%, respectively, and were 64% and 94%, respectively, for Ga-68 PSMA PET/CT. These values are even higher (76% and 97%) when Ga-68 PET imaging is combined with MRI, which is known to be superior to CT in soft-tissue imaging. This difference was found to be statistically significant between the successes of all three studies (p=0.03) (58). In the literature, there are new publications demonstrating the superiority of Ga-68 PSMA PET/MR combination only to MR (59,60). PET/MR studies, which can be performed in a single session of multiparametric MR, which is the anatomical imaging method with the highest accuracy and sensitivity in prostate cancer, with functional data provided by Ga-68 PSMA PET, are predicted to be used as a routine for imaging in prostate cancer patients in centers with PET/MRI facilities (61).

The most commonly used indication of Ga-68 PSMA PET/CT in prostate cancer is re-staging in patients with biochemical recurrence after primary treatment. The effectiveness of Ga-68 PSMA PET/CT at this stage has been demonstrated in numerous studies. In general, while the lesion detection rate is around 80%, there is a direct relationship between the serum PSA levels and the success of the examination. In a study, the detection rate was calculated as 58% in patients with serum PSA level of 0.2-1.0 ng/mL, 76% in patients with serum PSA level of 1-2 ng/mL and 95% in patients with serum PSA level of > 2 ng/mL. In a recent study, Ga-68 PSMA PET/CT was performed in 117 patients for biomechanical recurrence, and it was reported that Ga-68 PSMA PET/CT changed treatment strategy in 62-76% of patients and that 86% of these patients were given treatment for metastases detected by Ga-68 PET/CT (58). In these patients, Ga-68 PSMA PET/CT can show lymph node metastases in unexpected regions such as mesorectal, posterior pelvic region and supraclavicular region, and can detect occult metastases in lymph nodes below 1 cm that are not suspected radiologically (Figure 1). The sensitivity and specificity in lymph node assessment were 80% and 97%, respectively. It was found

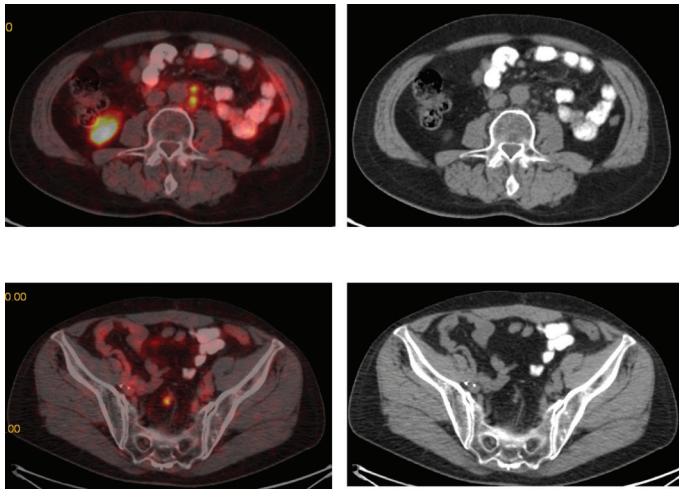

Figure 1. Millimetric lymph nodes in the paraaortic and presacral area in thoracoabdominopelvic CT of a 64-year-old patient who had pathology result of adenocarcinoma (Gleason 4+5) after radical prostatectomy and who developed PSA recurrence (tPSA=23.48 ng/mL) after radiotherapy. Ga-68 PSMA PET/CT showed intense pathological activity in these lymph nodes that did not reach the pathological dimension

to be more successful than bone scintigraphy in demonstrating bone lesions (62,63,64,65,66) (Table 3).

In a recently published meta-analysis, the effect of Ga-68 PSMA PET/CT on the treatment plan of the patients was investigated, and it was reported that Ga-68 PSMA PET/CT caused a change in the treatment plan in 54% of the patients, that the number of patients with systemic treatment decreased significantly and that the number of patients undergoing radiotherapy, focal therapy and surgery increased (67).

It has been reported that performing pre-treatment Ga-68 PSMA PET/CT for patients planned to receive primary or salvage RT may cause changes in the RT plan in 20-60% of patients. A better clinical response was found in patients with negative Ga-68 PSMA PET/CT before RT compared to positive results. A better response to RT is expected in patients with micrometastasis that is too small to be detected even with CT. It has been shown in a small number of small-scale studies that it was also successful in the evaluation of RT response in patients who developed biochemical recurrence after RT (68,69,70,71). It is also used to evaluate the response to treatment in patients under hormone therapy (Figure 2).

Although it has been introduced as a specific agent for prostate cancer, in recent years, incidental Ga-68 PSMA uptake has been reported in numerous benign and malign pathologies, except for prostate cancer. In patients diagnosed with prostate cancer, a secondary malignancy or benign events should be kept in mind in clinical interpretation when Ga-68 PSMA uptake is detected in atypical or unexpected localizations (72).

Although Ga-68 PSMA PET/CT is successful in showing occult lymph node metastases, <5 mm lymph nodes can be omitted with the partial volume effect under the PET resolution limit. Neuroendocrine differentiation was reported in prostate cancer as another cause of false negativity for Ga-68 PSMA PET/CT. In this group of patients, imaging with Ga-68 labeled DOTA peptides may be more appropriate (73,74,75,76,77).

C-11 Choline PET/CT

C-11 Choline is a PET agent that has been used for many years in prostate cancer, but there are many new studies reporting the superiority of Ga-68 PSMA PET/CT to C-11 Choline PET/CT in recurrent disease. While C-11 Choline seems to be advantageous because urinary excretion is less than that of Ga-68 PSMA, some lesions can be omitted because of its short half-life and need for imaging with short-term and rapid procedures. In addition, because it is a nonspecific agent compared to PSMA, numerous pathologies are known to cause false positivity (76,77).

F-18 Fluciclovin PET/CT

Anti-1-amino-3-F-18-fluorocyclobutane-1-carboxylic acid (Fluciclovin) is a synthetic amino acid analogue and is retained in prostate cancer through increased amino acid transport. It has been shown to be effective as a radiopharmaceutical in a large number of patients in prostate cancer and was approved by Food and Drug Administration (FDA) in 2016 for the re-staging of patients with PSA recurrence after primary treatment (78).

In a prospective clinical study comparing the role of Fluciclovin PET/CT, PET/MR and multiparametric MRI in the diagnosis of primary prostate cancer, it was shown that quantitative values obtained from Fluciclovin PET images were correlated with Gleason score but were not superior to multiparametric MR in detecting lesion. In this case, it was concluded that hybrid PET/MR images may be useful in prostate biopsies (79).

In a prospective study of 24 patients in whom biochemical response could not be obtained despite a primary treatment other than prostatectomy, the diagnostic power of Fluciclovin PET/CT was found to be significantly higher than multiparametric MRI (94.7% vs 31.6-36.8%). It was reported that this difference was particularly evident in the demonstration of extraprostatic disease, however, the sensitivity of Fluciclovin PET/CT for primary prostate tumor after treatment and the specificity of multiparametric MRI were higher (80).

In a prospective, multicentric study of the data of 213 patients, the efficacy of Fluciclovin PET/CT was investigated in the examination of biochemical recurrence after curative treatment, and in 57% of the patients, recurrence was shown in one or more foci with Flucyclovin PET/CT, and treatment approach was changed in 59% (81).

The most important advantage of Fluciclovin compared to other mentioned PET radiopharmaceuticals is that urinary excretion is significantly less. Thus, small foci present in the prostate bed and pelvic lymph nodes can be shown more easily. However, it has been reported that metastases in these areas may be omitted due to the relatively intense bone marrow and liver activity (78).

In a retrospective study demonstrating the efficacy of Fluciclovin PET/CT in 596 prostate cancer patients, it was reported as 41.4% even in patients with serum PSA levels <0.79 ng/mL. In a study comparing F-18 Fluciclovin PET/CT with Ga-68PSMA PET/CT in a small group of patients, Ga-68 PSMA PET/CT was positive in 7/10 patients, whereas Fluciclovin PET/CT was negative in 8/10 patients. While widespread disease could be demonstrated with Ga-68 PSMA PET/CT in 4/10 patients, it was reported that Fluciclovin PET/CT was negative in these patients (82).

Whole-Body Tc-99m MDP Bone Scintigraphy and 18F-NaF PET/CT

Tc-99m MDP and 18F-NaF are retained in bone lesions by binding to hydroxyapatite crystals. Although the mechanisms of retention are similar, Tc-99m MDP is a SPECT imaging agent used in conventional whole-body bone scintigraphy, and 18F-NaF is used as a positron spreading agent in PET/CT imaging. Generally, 18F-NaF is a more sensitive agent than Tc-99m MDP because of resolution superiority of PET imaging and its success in demonstrating both lytic and blastic lesions. However, because its relatively high cost, harder to obtain due to being a cyclotron product, and adequate and established success of Tc-99m MDP in demonstrating bone metastases in prostate cancer in the present protocols, 18F-NaF PET/CT is indicated only in suspected cases in this patient group. 18F-NaF PET/CT is

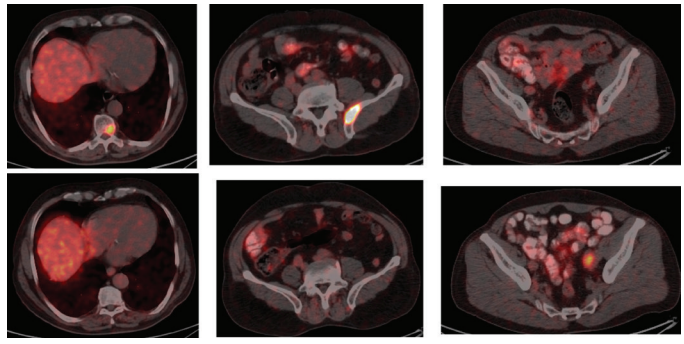

Figure 2. Pathological activity in multiple foci in skeletal system and left obturator lymph nodes in Ga-68 PSMA PET/CT performed in a 77-year-old patient with a prostate needle biopsy result compatible with acinar adenocarcinoma (Gleason 4+5) (First line). It was noted that activity in the bone lesions and lymph nodes defined in the Ga-68 PSMA PET/CT performed for the purpose of evaluating the response to treatment after hormoneotherapy decreased significantly (Second line)

Table 3. The diagnostic value of Ga-68 PSMA PET/CT and PET/MR in staging and re-staging of prostate tumors

Authors	Number of Patients	Indication	Method of screening	P/R	Sensitivity (%)	Specifity (%)	Accuracy Rate	Changes in treatment strategy (%)
Fendler WP et al. (53)	53	S	PET/MR	-	98	94	-	-
Soydal C et al. (57)	104	RS	PET/CT	R	92	80 (PSA <1.4 ng/mL) 90 (PSA <2 ng/mL)	-	-
Grubmüller B et al. (58)	117	RS	PET/CT and PET/MR	R	65 (PSA: 0.2-<0.5 ng/mL) 85.7 (PSA: 0.5-<1) 85.7 (PSA: 1-2) 100 (PSA ≥2)	-	-	74
Maurer t et al. (62)	130	RS	PET/CT and PET/MR	R	65.9	98.9	88.5	-
van Leeuwen PJ et al. (64)	30	RS	PET/CT	P	64	95	-	-
van Leeuwen PJ et al. (65)	70	RS	PET/CT	P	-	-	-	20
Bluemel C et al. (66)	45	RS	PET/CT	R	-	-	-	42.2
Calais J et al. (68)	101	RS	PET/CT	P	-	-	-	53
Habl G et al. (69)	100	RS	PET/CT and PET/MR	R	-	-	-	59

S: Staging, RS: Restaging, P: Prospective, RS: Retrospective, PSA: Prostate specific antigen

most commonly used for imaging before Ra-223 treatment and for evaluating post-treatment response (83,84,85,86).

Testicular Tumors

18F-FDG PET/CT

In the diagnosis of primary testicular tumors, the disease can be diagnosed correctly primarily by ultrasound and then MRI in almost all patients (87,88). It has been shown that metabolic imaging provides more accurate results in studies comparing 18F-FDG PET to conventional CT for staging in patients with primary testicular tumors. The success of detecting radiologically normal sized metastatic lymph nodes was reported as 70%, and this could significantly change the treatment approach in this patient group (89). The sensitivity in seminomatous germ cell tumors (SGCT) is slightly better in comparison with nonseminomatous germ cell tumors (NSGCT) (90-92% vs 77-96%) (90).

In the presence of metastatic disease, residual masses may continue in 55-80% of patients after chemotherapy. In particular, 11-37% of the masses >3 cm can still have live tumor tissue in seminoma cases. Surgical interventions after chemotherapy may be challenging and morbid due to fibrosis. For this reason, it is important to distinguish between live tumor tissue and fibrosis before surgery. 18F-FDG PET/CT has been used for many years for this indication and there are studies in the literature about its role in postoperative follow-up of testicular tumors (91,92,93). In a meta-analysis, for this purpose, sensitivity was reported as 78%, specificity as 86%, and overall accuracy rate as 84% in SGCT. 18F-FDG PET/CT has been shown to be more successful in lesions greater than three centimeters (94). The success in predicting live tumor tissue decreases to 56% in NSGCT (95). Since residual masses may contain up to 40% mature teratoma in this patient group, necrosis-live tissue distinction may not be clearly performed with 18F-FDG PET/CT (96). Prospective, large-scale studies are needed to clarify the role of 18F-FDG PET/CT in NSGCT (Table 4).

18F-FLT PET/CT

The fact that 18F-FDG uptake is observed in false positive lesions in inflammatory lesions has led to the hypothesis that more accurate results can be obtained with other tumor-specific agents in the differentiation of live tumor-necrosis or fibrosis. In a small-scale study of 18F-FLT, a cell proliferation marker, its success in evaluating early response to treatment was investigated. Although false positivity rates could be reduced by 18F-FLT in this study, the presence of live tumor tissue in residual masses could not be ruled out with 18F-FLT, as negative predictive value was not high enough (97).

Conclusion

• 18F-FDG PET/CT in RCC is successful in staging in high-risk disease and demonstrating response to treatment in patients with metastatic disease. However, there is a need for further studies on routine use. Tc-99m MIBI SPECT/CT has high sensitivity and specificity in the malignant-benign differentiation of indeterminate renal masses.

• 18F-FDG PET/CT has a role in staging and re-staging of muscle-invasive bladder cancer, and can provide an idea about prognosis.

• Although not involved in the metabolic characterization of primary scrotal masses, 18F-FDG PET/CT is useful in the staging, restaging and follow-up of testicular tumors, especially in the evaluation of seminoma patients with a >3 cm residual retroperitoneal lesion after treatment. The role of imaging with F-18 and C-11 labeled other radiopharmaceuticals in order to reduce the rate of false negativity associated with physiological renal clearance of 18F-FDG has not yet been elucidated.

• Ga-68 PSMA PET/CT in prostate cancer has high sensitivity in every stage, especially in patients with biochemical recurrence and its use is becoming more common. In addition, conventional bone scintigraphy with Tc-99m MDP is still sufficient for imaging bone metastases. 18F-NaF PET/CT can be used as a more expensive but more sensitive alternative in selective cases, such as patients who are scheduled for treatment with Ra-223.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.A., Y.Ü., Design: M.A., Y.Ü., Data Collection or Processing: M.A., Y.Ü., Analysis or Interpretation: M.A., Y.Ü., Literature Search: M.A., Y.Ü., Writing: M.A., Y.Ü.

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

Table 4. Diagnostic value of 18F-FDG PET and PET/CT in staging and re-staging of testicular tumors

Authors	Number of Patients	P/R	Screening	Indication	Patology (S/NS)	Sensitivity (%)	Specifity (%)	Accuracy Rate (%)
Lassen U et al. (89)	46	R	PET	RS	NS	70	100	93
Ambrosini V et al. (90)	121	R	PET/CT	RS	S ve NS	S: 92 NS:77	S: 84 NS: 95	-
Oechsle K et al. (95)	121	P	-	RS	NS	70	48	-
Bachner et al. (91)	127	R	PET	RS	S	67	82	-
Siekiera et al. (92)	37	R	PET/CT	RS	S	100	94	-
Hinz S et al. (93)	20	P	PET	RS	S	100	47	-

P: Prospective, R: Retrospective, RS: Restaging S: Seminomatous, NS: Non-seminomatous tumor

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