NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL GENETICS
M.Sc. PROGRAM IN MEDICAL BIOLOGY AND GENETICS

ASSESSMENT OF KI-67 LABELING INDEX IN PROSTATE CANCER RISK CLASSIFICATION

M.Sc. THESIS

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Nicosia
November, 2022

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Approval

We certify that we have read the thesis submitted by Atefeh Jafari entitled **"Assessment of Ki-67 Labeling Index in Prostate Cancer Risk Classification"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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Declaration

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Faculty of Medicine, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Atefeh Jafari

14/11/2022

Acknowledgements

First, I would like to express my sincere gratitude to my esteemed supervisor Asst. Prof. Dr. Fikret Dirilenoglu for his unconditional academic and professional support in every step of this thesis. I would like to thank the experienced and skilled technicians of the Department of Pathology, Near East University, Faculty of Medicine including Busra Ozciger, Hanife Eriman for their technical assistance during slide preparation and immunohistochemical staining procedures. In addition, I would like to thank Dr. Zeliha Kocaismail for her help in finding clinical information of our study cases. Moreover, I sincerely appreciate my beloved family and well-experienced teachers to support and guide me to broaden the horizons of my knowledge.

Atefeh Jafari

Abstract

Assessment of Ki-67 Labeling Index in Prostate Cancer Risk Classification

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November 2022, 60 pages

Introduction: Prostate cancer (PCA) is the most common malignancy and the second leading cause of cancer fatalities in Europe and the United States. Although more than 95% of PCA cases are histologically acinar adenocarcinomas, they are biologically and clinically heterogeneous. Hence, novel biomarkers have been investigated for better prognostic stratification. Ki-67 is a powerful marker of cell proliferation which is assessed by pathologists in various indications using an immunohistochemical MIB1 stain. Ki-67 labeling index (LI), meaning the fraction of Ki-67-positive cells, has shown promising results in PCA prognostication. Our aim is to assess the relationship between Ki-67 LI and the risk classification systems for PCA patients, including D'Amico and CAPRA, using an objective and reproducible Ki-67 LI counting method.

Methodology: We retrospectively searched and included the cases with a pathological diagnosis of acinar adenocarcinoma of the prostate from core biopsy samples. We recorded the clinical and pathological information including age, preoperative prostate-specific antigen (PSA) level, percentage of positive biopsy cores, Gleason grade, ISUP grade group, and clinical T stage. Then, we stratified the cases into low-, intermediate-, and high-risk groups according to the D'Amico and CAPRA risk classifications. Ki-67 immunostaining was performed on a representative block from each case. We calculated the Ki-67 LI by applying three counting methods. The method of choice in our clinicopathological analyses was manual counting. We utilized free mobile software (CFU.Ai) to ease the counting of cells. We performed the statistical analyses using SPSS Statistics 22.0 package program. A value of $p < 0.05$ was considered significant.

Results: We included 92 cases with sufficient clinical information and pathological material in our study. The median age of the patients was 70. The median PSA level

was 9,8 ng/ml. Most cases (70.6%) were in lower ISUP grade groups (less than 4). Most cases (84.8%) were in the stages T1 and T2. There was a strong positive correlation among all counting methods ($p < 0.001$). We found a strong positive correlation between Ki-67 LI and Gleason score, ISUP grade group, clinical stage, and both risk classifications ($p < 0.001$). The cut-off value for Ki-67 LI (manual counting) was significant at 12%. We found that high Ki-67 LI was significantly associated with high Gleason score, ISUP grade group, clinical stage, and clinical risk ($p < 0.001$). There was no statistically significant association between the homogeneity of Ki-67 staining and clinicopathologic factors.

Discussion: Previous studies have shown the prognostic importance of Ki-67 LI in PCA, although there was limited information about the relationship between Ki-67 LI and PCA risk classifications. We used the manual Ki-67 counting method, which is a highly objective, accurate, and reproducible technique. We showed that there is a strong positive correlation between Ki-67 LI and both risk classifications ($p < 0.001$). In addition, our cut-off value (12%) was able to divide most of the cases into high-risk and low-risk. Using two other Ki-67 counting methods did not affect this powerful association. Our results support that Ki-67 can be used as a supportive or surrogate tool to determine the risk status of PCA cases and in choosing a more personalized treatment approach.

***Keywords**:* Ki-67 labeling index, prostate cancer, risk classification, acinar adenocarcinoma

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List of Abbreviations

AA:	Acinar adenocarcinoma
ASTRO:	American Society for Radiation Oncology
AUA:	American Urology Association
CAPRA:	Cancer of the Prostate Risk Assessment
cT:	Clinical stage
DRE:	Digital rectal exam
GG:	Grade group
GS:	Gleason score
IHC:	Immunohistochemical
ISUP:	International Society of Urological Pathology
PCA**:**	Prostate cancer
PSA:	Prostate-specific antigen
ROC:	Receiver operating characteristics
SUO:	Society of Urologic Oncology
WHO:	World Health Organization

CHAPTER I

Introduction

Prostate cancer (PCA) is the most common cancer and the second leading cause of cancer fatalities in Europe and the United States (Rawla, 2019). A total of 1,414,000 new PCA cases and 375,304 deaths were anticipated in 2020 (L. Wang et al., 2022). Although more than 95% of PCA cases are histologically acinar adenocarcinomas (AA), they are biologically and clinically heterogenous (Humphrey, 2017). Accordingly, the established prognostic factors alone could not provide a sufficient risk assessment of PCA patients. Using a combination of several powerful prognostic factors, a few risk classification systems have been established, including the D'Amico and University of California, San Francisco – Cancer of the Prostate Risk Assessment (CAPRA) score (M. R. Cooperberg, Pasta, et al., 2005; G. D'Amico & Luca, 1997). These systems have been helpful to estimate clinical outcomes by stratifying cases into low-, intermediate-, and high-risk categories. Nevertheless, novel biomarker investigations are ongoing to better stratify PCAs which would be incorporated into these risk classification systems and provide improved personalized management of patients.

Scholzen and Gerdes discovered Ki-67 in the early 1980s (Scholzen & Gerdes, 2000). *MKI-67* gene encodes Ki-67 which is expressed during cell cycle's active phases (G1, S, G2, M), while, not in resting (G0). The levels of Ki-67 drastically decrease at the end of mitosis. Hence, Ki-67 is known as a powerful marker of cell proliferation. MIB1, a monoclonal antibody, was developed to detect the Ki-67 labeling index (LI) meaning the fraction of Ki-67-positive cells (L. T. Li et al., 2015). Since then, Ki-67 LI has been one the most widely adopted immunohistochemical (IHC) markers in pathology practice in distinguishing neoplastic lesions from nonneoplastic lesions, e.g., atrophy vs dysplasia of the uterine cervix, grading of tumors, e.g. the lung and gastrointestinal neuroendocrine

tumors, and in prognostication, e.g., breast carcinomas (Hirabayashi et al., 2013; Kamal, 2022; Nielsen et al., 2021a; Pelosi et al., 2014).

There has been accumulating evidence on the prognostic significance of Ki-67 in PCA. Ki-67 LI was found to be significantly associated with clinical outcomes, including biochemical failure-free survival, disease-free survival, disease-specific survival, rate of distant metastasis, and overall survival shown in a meta-analysis of 21 studies comprising 5419 patients (Berlin et al., 2017). Its association with histopathologic prognostic factors, i.e., Gleason score (GS), ISUP grade group (GG), extracapsular extension, and seminal vesicle invasion was also found to be significant in several studies (Fantony et al., 2018b; Richardsen et al., 2017b; Ronaldo Maia1; GabRiel aRantes dos santos2, 2022; Tretiakova et al., 2016b) . Therefore, Ki-67 is one of the most promising markers to be adopted in PCA.

The significance of Ki-67 LI in risk classification systems for PCA has not been well-studied. Furthermore, the adoption of Ki-67 LI in PCA has been hampered by different cut-off value selections due to using subjective counting methods (Richardsen et al., 2017b). Our main goal is to evaluate the significance of the Ki-67 LI, determined by an objective counting technique, in clinical risk classification systems, including D'Amico and CAPRA. In addition, we aimed to correlate the Ki-67 LI with clinical and pathological factors that are associated with PCA prognosis.

CHAPTER II

Literature Review

1. Epidemiology and Aetiology of Prostate Cancer*:*

Prostate cancer (PCA) is the most common malignancy and the second leading cause of cancer fatalities in Europe and the United States (Dyba et al., 2021; Siegel et al., 2022). A total of 1,414,000 new PCA cases and 375,304 deaths were anticipated in 2020 (L. Wang et al., 2022). The risk of PCA increases with age, > 85% of newly diagnosed individuals are over 60 years old. The variation in PCA prevalence among geographic regions has raised the possibility of a genetic basis. Certain ethnic groups are associated with higher PCA rates. For instance, compared to white populations, those of African or Caribbean heritage have a threefold higher relative risk of PCA. (Rebello et al., 2021). The occurrence of PCA is more common in high-income countries. Although people of Asian heritage who live in Asia have a lower risk of PCA than white men who live in the USA, when they come to the USA, their risk rises. These findings suggest that a western lifestyle and dietary factors seem to underlie the increased PCA risk (Rebello et al., 2021; Zavala et al., 2021). Other environmental factors suggested in the aetiology include inflammation, infections, and environmental exposure to some chemicals or ionizing radiation (Rawla, 2019).

2. Clinical Features of Prostate Cancer Patients

Most PCA cases are not symptomatic in countries where prostate-specific antigen (PSA) screening is available (Rawla, 2019; Wollersheim et al., 2021). A patient is symptomatic usually after a locally-advanced disease. The symptoms in locally-advanced stage include urinary frequency, difficulty urinating, acute urinary retention,

haematuria, haematospermia, and impotence. In highly-advanced local diseases, rectal invasion, priapism, and uraemia may occur. In the late stage, metastatic disease may result in bone pain, pathological fractures, oedema of the lower extremities, and neurological complaints (Holger Moch, 2016).

3. Diagnostic Evaluation of Prostate Cancer Patients:

The suspicion of prostatic carcinoma relies on elevated PSA level and/or digital rectal exam (DRE) (Descotes, 2019; Grubb et al., 2008; Szymańska & Hainaut, 2018). PCAs are often missed on DRE or detected in later stages, therefore, PSA screening is used as a more sensitive test (Grubb et al., 2008; Holger Moch, 2016). PSA levels are often above 2 ng/ml in PCA patients (Holger Moch, 2016; Mistry & Cable, 2003). However, an increase in PSA levels is not specific since it may be found high in benign prostatic diseases or due to mechanical manipulations (Bratt & Lilja, 2015; Holger Moch, 2016; Stephan et al., 2014). Nevertheless, PCA is very uncommon to occur in those with PSA levels under 1 ng/ml. (Aus et al., 2005; Holger Moch, 2016).

Other markers have been studied to improve deficiencies of PSA. PSA derivatives, such as PSA density, PSA doubling time, PSA velocity, and age- and race-specific PSA reference ranges have shown some improvements in the specificity (Holger Moch, 2016; Loeb & Catalona, 2007; Lopez-Beltran et al., 2012; Vickers et al., 2009). PSA is in a complex form with free-PSA and a few proteins (Holger Moch, 2016; Stephan et al., 2014). Low values (< 20%) of free-PSA have been found to be more sensitive in detecting PCA (Catalona et al., 1998; Holger Moch, 2016). *TMPRSS2-ERG* gene fusion is present in about 50% of PCA cases and can be identified in urine samples (Esgueva et al., 2010; Holger Moch, 2016; Perner et al., 2006). A non-coding mRNA called PCA3 is overexpressed in PCAs, however, issues in cut-off value determination have limited its use over PSA test (Hessels et al., 2003; Holger Moch, 2016; Roobol, 2011; Truong et

al., 2013). Combining serum and urine biomarkers in diagnosis (serum PSA + urinary PCA3 + urinary TMPRSS2-ERG gene fusion) appears to be more sensitive and specific (Holger Moch, 2016; McGrath et al., 2016; Salami et al., 2013; Stephan et al., 2013).

There are various imaging techniques aiding in PCA diagnosis. Transrectal prostate ultrasound is the first technique recommended in diagnostic settings when PCA is suspected based on a high PSA level or an abnormal DRE. The magnetic resonance imaging technique is used to locate PCA and determine their size and extent of invasion. Histopathologic confirmation of PCA is made either by a core biopsy or transurethral resection of the prostate or prostatectomy (Descotes, 2019; Holger Moch, 2016; Szymańska & Hainaut, 2018).

4. Histopathology of Prostate Cancer*:*

According to 2016 World Health Organization (WHO) classification, there are a variety of PCA including epithelial, neuroendocrine, mesenchymal, haematolymphoid, miscellaneous and metastatic tumors (Humphrey et al., 2016). The most common histological type of PCA is AA, which represents over 95% of PCAs. It is made up of neoplastic prostatic epithelial cells showing secretory differentiation arranged in various patterns, including glands, cords, single cells, and sheets. Lack of basal cells is a typical finding (Han et al., 2021; Holger Moch, 2016; Magi-Galluzzi, 2018; Swerdlow et al., 2016; Szymańska & Hainaut, 2018).

A constellation of several findings is required for the histopathological diagnosis of PCA, including architectural, nuclear, cytoplasmic features, intraluminal contents, stromal response, and lack of basal cells (Baig et al., 2015; Beheshti et al., 2018; Magi-Galluzzi, 2018). None of these findings should be used alone and the mimickers of cancer must be ruled out to reach the diagnosis. On the other hand, there are three histological features that are specific to PCA including glomerulations, perineural invasion, and mucinous

fibroplasia (also known as collagenous micronodules)(Humphrey, 2017; Magi-Galluzzi, 2018).

Immunohistochemical (IHC) examination may be needed in certain indications, such as diagnosing limited (minimal) adenocarcinoma on core needle biopsy, discrimination of poorly differentiated prostatic carcinoma from carcinomas or mimicker lesions of adjacent organs, and diagnosis of metastatic tumors (Holger Moch, 2016). Basal cells are highlighted by high-molecular-weight cytokeratin and p63/p40. (Holger Moch, 2016; Sailer et al., 2013). Lack of staining strongly suggests a malignant infiltration. AMACR is a sensitive stain for neoplastic prostatic glands in 80-100% of the cases (Holger Moch, 2016). It should be combined with basal cell markers to exclude non-invasive prostatic intraepithelial neoplasia. ERG is a specific marker for neoplastic prostatic glands, however, has a sensitivity of about 50% (Andrews & Humphrey, 2014; Holger Moch, 2016). In poorly differentiated PCAs, highly sensitive IHC markers including PSA, prostatic acid phosphatase, prostein, and NKX3.1 can be used (Epstein et al., 2014; Holger Moch, 2016; Huang et al., 2018). Other organ-specific markers, such as GATA3 and CDX-2, can be utilized in the differential diagnosis of metastatic tumors (Epstein et al., 2014; Holger Moch, 2016).

4a. Grading in Prostate Cancer*:*

The Gleason grading has long been used as the gold standard grading system to determine the differentiation and aggressiveness of PCA (Epstein et al., 2017). It relies solely on the architectural patterns of PCA. The grade patterns range from 1 (the most differentiated) to 5 (the least differentiated). A GS is assigned for each case based on the combination of the two most frequent grade patterns. (for example 3 + 4 = Score 7). GS 2 to 5 are not reported due to poor reproducibility, poor concordance with the patterns in radical specimens, and clinical misjudgement. Gleason scoring provided powerful prognostic

stratification of patients with a few deficiencies. GS 7 includes both 3 + 4 and 4 + 3, however, clinical management and prognosis were different in these groups (Barakzai, 2019). The scoring scale seems to range from 2 to 10, however, the lowest score begins from 6 which distress patients as the score places in the middle of the scale. To overcome these issues, a modified version of the Gleason system was developed by the International Society of Urological Pathologists (ISUP) in 2014 **(Table 1)**. This new system has provided more accurate, simplified (1 to 5), and rational stratification of PCAs (Epstein et al. 2016).

Table 1. *Comparison of the Gleason Score and ISUP Grade Group systems.*

Gleason Patterns	Gleason Score	Grade Group
3 + 3	6	1
3 + 4	7	2
4 + 3	7	3
4 + 4 or 3 + 5 or 5 + 3	8	4
4 + 5 or 5 + 4	9	5
5 + 5	10	5

4a. **Risk Classifications in Prostate Cancer***:*

For prognosis and treatment, localized PCAs should be classified as low-, intermediate-, or high-risk (Parker et al. 2020). A few classification systems have been used to assess PCA risk, including the D'Amico and CAPRA.

D'Amico Risk Classification System

This system was developed by D'Amico et al. in 1988 to estimate the risk of biochemical failure in PCA patients who underwent radical prostatectomy and external-beam radiotherapy (G. D'Amico & Luca, 1997). The calculation of risk score is made using PSA level, GS, and clinical stage (cT; according to DRE findings). Patients with cT1c-cT2a and PSA levels of 10 ng/mL or less, along with biopsy GS of 6 or less, are considered low risk; those with cT2b

or PSA levels of 10.1 to 20 ng/mL, or biopsy GSs of 7, are considered intermediate risk; and those with cT2c or PSA levels greater than 20 ng/mL, or biopsy GSs 8 to 10, are considered high (Boorjian et al., 2008; Hernandez et al., 2007b).

In 2018, a modified version of D'Amico system was suggested by the American Urology Association/American Society for Radiation Oncology/ Society of Urologic Oncology (AUA/ASTRO/SUO) guideline. The parameters used in this system include PSA level, PSA density, GG, cT, biopsy core positivity, and percentage of positive biopsy core (Sanda et al. 2018).

CAPRA Risk Classification System

It determines a patient's risk of metastasis, cancer-specific fatality, and overall mortality across numerous treatment modalities. The system is based on the following factors: age at diagnosis, PSA at diagnosis, biopsy GS, cT, and the percentage of biopsy cores positive for cancer (Cooperberg, Hilton, and Carroll 2011; May et al. 2007). After entering variables in the CAPRA scoring tool, a total score is assigned and categories are determined as follows: low-risk (between 0-2 scores), intermediate-risk (3-5 scores), and high-risk (6-10 scores) (**Table 2**).

Table 2. *CAPRA risk classification system*

Parameters	**Stage**	**Points**
Age at diagnosis	<50	0
	≥50	1
PSA level at diagnosis, ng/mL	0-6	0
	6.1-10	1
	10.1-20	2
	20.1-30	3
	Above 30	4
Clinical stage	T1 or T2	0
	T3a	1
Gleason score	No pattern	0

	4 or 5 Secondary pattern	1
	4 or 5 Primary pattern 4 or 5	3
Percentage of biopsy cores positive for cancer	less than 34%	0
	34% and above	1

1. **Ki-67**

5a. ***MKI-67 Gene***

The city of Kiel (Ki), where Gerdes and his team worked in the pathology and biochemistry departments of the university, is where the term "Ki-67" originates, and "67" refers to the 96-well plate's original clone. (Klöppel and la Rosa 2018). On chromosome 10q25-ter, it is a continuous sequence of 29,965 bp made up of 15 exons with sizes ranging from 67 to 6845 bp and 14 introns with sizes ranging from 87 to 3569 bp. The core of the gene contains 16 similar 366-bp regions in exon 13. A 264-bp 3′ region and a 74-bp 5′ region make up the entire gene (L. T. Li et al., 2015). In humans, the MKI-67 gene encodes the Ki-67 protein (Sun and Kaufman 2018).

5b. Ki-67 Protein*:*

Scholzen and Gerdes discovered the Ki-67 antigen for the first time in the early 1980s. It is expressed in two protein isoforms with molecular weights of 345 and 395 kDa (Scholzen & Gerdes, 2000). The Ki-67 protein has a half-life of only ~1–1.5 h. Ki-67 has a strong positive net charge. It allows the peri-chromosomal layer to prevent mitotic chromosomes from binding together. Like surfactants that scatter particles or phase-separated liquid droplets in solvents, Ki-67 creates a steric and electrical barrier. (Cuylen et al., 2016). New research also indicates that the Ki-67 and cohesion complexes serve distinct roles in the structural integrity of mitotic chromosomes, with the co-depletion of Ki-67 leading to condensing, which results in the formation of an amorphous slime ball from the chromosomes (Takagi et al., 2018).

Ki-67 is expressed during all active phases of the cell cycle (G1, S, G2, M), while, not in resting cells (G0). At the end of mitosis (between anaphase and telophase), Ki-67 levels drastically decrease (Li et al. 2015). Ki-67-depleted cells fail to assemble metaphase plates and almost never proceeds to anaphase (Cuylen et al., 2016). Ki-67 depletion inhibits S phase entrance in some cell culture systems (Uxa et al. 2021). The quantity of Ki-67 is precisely controlled by the balance between synthesis and degradation during any stage of the cell cycle. Its structure suggests that proteolytic pathways are able to recognize its expression, including cyclin B/cyclin-dependent kinase 2. Ki-67 appears to be involved in multiple stages of carcinogenesis (Uxa et al. 2021). In malignant tumours, Ki-67 expression is linked to intrinsic cell proliferation, enabling it to be used as a marker of tumour aggressiveness (Li et al. 2015).

5c. Ki-67 Test (Labelling Index (LI) Measured by Immunohistochemical Staining*:*

The standard antibody used in detecting the Ki-67 antigen is called MIB1. The Ki-67 LI can be measured by counting how many tumor cells are stained by the antibody, with nuclear staining determining cell positivity (Greenberg et al. 2001). Ki-67 LI has been a test in different tumour types including breast cancer, neuroendocrine tumor, lymphoma, sarcoma, multiple myeloma, soft tissue sarcoma, and oral squamous cell carcinoma (Broyde et al., 2009; Gadbail et al., 2021; Kim et al., 2007; Remnant et al., 2021; Zaiem et al., 2020). In numerous tumor types, the prognostic importance of Ki-67 LI has been demonstrated. (Nielsen et al., 2021b; Tong et al., 2020; D. Wei et al., 2018).

Detection of Ki-67 may potentially be utilized in targeted cancer therapies such as Ki-67-antisense nucleotide, Anti-Ki-67 peptide nucleic acid, RNA interference targeting Ki-67, oncolytic adenoviral-mediated Ki-67-siRNA, oncolytic adenovirus targeting

both Ki-67 and telomerase, Ki-67 promoter-controlled cancer gene therapy. Additionally, the Ki-67 promoter has proven to be a desirable target for siRNA or therapeutic gene expression in cancer cells (Yang et al. 2018).

5. **Other Promising Biomarkers in Prostate Cancer***:*

In PCA, novel promising molecular biomarkers are emerging that are associated with aggressiveness and therapy prediction.

Hypoxic mediators have significant functions in aggressive tumors and metastasis. Hypoxia-inducible factor 1 (HIF-1α) is an essential regulator of the tumor hypoxia response. In PCa, elevated HIF-1α has been related to the phenotype of aggressive tumors and poor prognosis. Inhibitors of HIF-1 have been shown to have anti-cancer effects on PCA. (Deep et al., 2017; Fraga et al., 2015; Lee et al., 2016; Ma et al., 2018; Mitani et al., 2012; Ranasinghe et al., 2014). There was a notable association between GS and HIF-1 expression (Ma et al., 2018). In the hypoxic conditions, HIF-1α operates the expression of downstream genes, including vascular endothelial growth factor (VEGF). VEGF is a substantial angiogenic factor (Al-Ubaidi et al., 2012; Ma et al., 2018). Angiogenic factors play a critical role in the growth and invasion of PCA (Ma et al., 2018). High expression of VEGF in PCa was found to be associated with poor overall survival (Ma et al., 2018; Tomić et al., 2012; K. Wang et al., 2012)

PTEN is a protein and lipid phosphatase which acts against the oncogenic PI3K/AKT signalling pathway. In early PCA, loss of PTEN usually occurs due to genomic deletion, although genomic rearrangements and rare truncation mutations resulting in PTEN inactivation have been defined (Jamaspishvili et al., 2018; Lotan et al., 2020). It was shown that PTEN loss is notably associated with the risk of fatality in PCA (Lotan et al., 2020).

Androgen receptor splice variant 7 (AR-V7) is a significant oncogenic driver and active AR isoform without needing ligand

binding. Many patients attain an AR-V7, fundamentally activated and lack the ligand-binding region. This alternation allows it to remain in the nucleus as a transcription factor suppressing vital tumor suppressor genes even in the lack of the ligand (Sobhani et al., 2021). Both healthy and cancerous prostatic tissues have been examined for AR-V7 expression. It was seldom detected in early PCA (1%) but found to be expressed more than 75% of metastatic cases following androgen-deprivation therapy. This marker may be targeted by niclosamide and TAS3681 (novel medications) (Sharp et al., 2018; Sobhani et al., 2021).

A recent study screened 48 protein biomarkers using a combination of the ELISA and Mass Spectrometry-Guided Immunoassay Development methods. Machine learning-based analysis showed that testing two extracellular matrix proteins, fibronectin and vitronectin, together with PSA provided a better prediction of biochemical recurrence-free survival compared to other gold standard prediction methods, such as PSA alone or combined PSA and GS (Goetze et al. 2022).

Several biomarkers investigated in PCA have been found to be at higher levels including interleukin-10, monocyte chemoattractant protein-1, soluble tumor necrosis factor receptor-1, endothelial growth factor, VEGF, C-reactive protein, and D-dimer. In comparison to tPSA alone, the combination of EGF, log10 IL-8, log10 MCP-1, and log10 tPSA greatly enhanced PCA prediction (McNally et al., 2022).

CHAPTER III

Methodology

Case Selection:

The patients who underwent systematic prostate core needle biopsies were retrospectively identified using the Near East hospital information system at the Department of Pathology from 2015 to 2022. All the cases diagnosed as AA have been selected. Clinical information of the selected cases was recorded from the databases of the Departments of Pathology and Urology. The cases with sufficient pathology material and clinical information including age, preoperative PSA level, percentage of positive biopsy cores, GS, ISUP GG, and cT were included. The cases in GGs 1, 2, and 3 are regarded as low grade; and GGs 4 and 5 as high grade. The cases without adequate tumor area after immunostaining or with incomplete clinical information were excluded.

All the slides belonging to the selected cases were retrieved from the pathology archive. From each selected case, one biopsy sample with the highest tissue quality and sufficient tumor area was chosen with microscopic examination.

Risk Classifications:

The selected cases were stratified into low, intermediate, and high risk according to the D'Amico risk classification and the CAPRA systems.

Immunohistochemical Staining for Ki-67*:*

A 4-micrometer section from each formalin-fixed, paraffin-embedded tissue block was obtained. Tonsil was used as a positive tissue control. Immunohistochemical staining for Ki-67 was applied using Ventana CONFIRM™ anti-Ki-67 (30-9) Rabbit Monoclonal

Primary Antibody on a Ventana automated slide stainer according to the streptavidin-biotin immunoperoxidase technique. Positive staining was defined as brown nuclear immunoreactivity.

Evaluation of Ki-67 Labeling Index (LI):

To determine Ki-67 LI, three counting methods were used, including Ki-67 LI in the entire biopsy via eyeballing (overall e-Ki-67 LI), in hot spot area via eyeballing on 400X magnification (hot spot e-Ki-67 LI), and manual counting from a screenshot of the hot spot area on 400X magnification (manual Ki-67 LI). In manually counting Ki-67 positive cells and all tumor cells, we utilized free mobile software called "CFU.Ai". Once an image is uploaded, Ki-67-positive tumor cells are automatically marked. Unidentified positive cells can be marked or misidentified cells can be easily deselected by tapping on the screen. Any cell with brown nuclear staining was considered positive (Kinra & Malik, 2020b). After identifying the Ki-67 positive cells, all tumor cells are selected. Then, the ratio of Ki-67-positive cells to all tumor cells are calculated. The software automatically counts the number of selected cells in the image.

The manual Ki-67 LI method was determined as the standard due to its high objectivity, accuracy, and reproducibility. Using receiver operating characteristics (ROC) curve analyses, cut-off value was determined as 12% based on PCA risk classification systems. According to the cut-off value, the cases were divided into "Ki-67-low" and "Ki-67-high" groups.

In addition, we assessed and recorded the homogeneity of Ki-67 staining in the entire biopsy via eyeballing.

Statistical Analysis

All statistical analyses were performed using SPSS Statistics 22.0 package program (IBM Corp., Armonk, New York, USA). The frequencies of clinical and histological variables were presented using

cross-tabulations. A two-sided Fisher's Chi-Square exact test for Rx tables was applied to compare the differences between the groups for categorical variables. The normal distribution of variables was examined visually (histogram and probability plots) and with analytical methods (Shapiro-Wilk tests). If at least one of the variables was not normally distributed or ordinal, the correlation coefficients and statistical significance were calculated by the Spearman test for inter-variable relationships. A value of $p < 0.05$ was considered significant.

CHAPTER IV

Results

This study was approved by the Noninterventional Ethics Board of the Near East University with decision number 1539 on 31 March 2022.

Patients and Clinicopathological Characteristics

Using the hospital information system, we identified 116 cases who underwent prostate biopsy and were histopathologically diagnosed as AA. Twenty-four cases were excluded for the following reasons: a- nine cases lacking tissue blocks b- seven cases with no remaining tumor cells after sectioning c- five cases with abundant inflammation and only a few tumour cells d- three cases without sufficient clinical information. Ninety-two cases with sufficient clinical information and pathological material were included in our study.

The median age of the patients was 70 (ranging from 44 to 87). The median PSA level was 9,8 ng/ml (ranging from 0.87 to 3625) **(Table 3)**. Forty-three (46.7%) patients were in cT1, 35 (38%) patients were in cT2, and 14 (15.2%) patients were in cT3. The number of cases in each GS group and the ISUP GG was shown in **Table 4.1** and **Table 4.2**.

Table 3. *Descriptive statistics of the patients*.

Descriptive Statistics (n = 92)					
	Range	Minimum	Maximum	Median	Std. Deviation
Age	43	44	87	70	8,520
Preoperative PSA Level	3624,13	,87	3625,00	9,8	420,61401
Percentage of Positive Cores	93,71	,04	93,75	0,5	9,72651

PSA: Prostate-specific antigen

Table 4.1. *The number of cases in Gleason score groups.*

		n	%
Total Gleason Score	6	29	31,5

	7	36	39,1
	8	12	13
	9	14	15,2
	10	1	1,1
Total		92	100

Table 4.2. *The number of cases in each ISUP grade group.*

		n	%
ISUP Grade Group	1	29	31,5
	2	24	26,1
	3	12	13
	4	12	13
	5	15	16,3
Total		92	100

The number of cases in each of CAPRA and D'Amico risk gruops were given in **Table 5.** We found a significant positive correlation between the CAPRA and D'Amico risk classification systems (r = 82%, $p < 0.001$).

Table 5. *The number of cases in each of CAPRA and D'Amico risk groups.*

Risk Groups	**CAPRA (n, %)**	**D'Amico (n, %)**
Low risk	21 (22.82)	20 (21.73)
Intermediate Risk	36 (39.13)	31 (33.69)
High Risk	35 (38.04)	41 (44.56)
Total	92	92

Determination of the Ki-67 Cut-off Value for Prostate Cancer Risk Classification

We used the ROC curve analysis to find the most significant Ki-67 LI cut-off values in determining the risk category of PCA cases with optimum sensitivity and specificity. We applied and assessed the ROC curve analysis for each of the Ki-67 counting methods. The graph (**Figure 1**) and tables (**Table 6.1, 6.2**) demonstrate the area under the curve, sensitivity, specificity, p values, and cut-off values for each of the Ki-67 counting

methods. We selected 12% cut-off value determined by manual Ki-67 LI counting method in our clinicopathological analyses ($p < 0.001$).

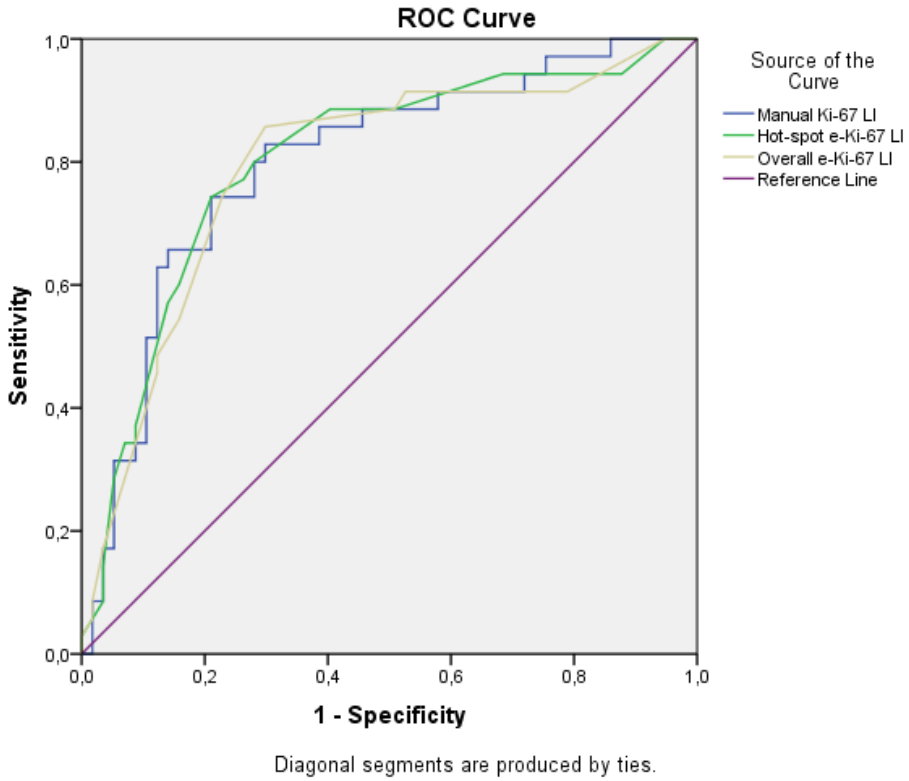

Figure 1 – The graph demonstrates that x-axis equals to 1 – specificity [= false positive fraction = FP (false positive) / (FP+TN (true negative)]. The y-axis equals to sensitivity (= true positive fraction = TP (true positive)/ (TP+FN (false negative)). To determine the most significant cut-off value, the ROC curve point closest to the left-upper corner of the unit square is selected as the best cut-off point (Habibzadeh, Habibzadeh, and Yadollahie 2016).

Table 6.1, **6.2** *The results of the ROC curve analyses show the optimum Ki-67 cut-off value according to CAPRA and D'Amico risk classifications.*

		CAPRA			
Ki-67 counting method	AUC (95%CI)	Cut-off	p-value	Sensitivity (%)	Specificity (%)
Manual Ki-67 LI	0.8 (0.704-0.895)	12.85	< 0.001	74.3	73.7
Hot-spot e-Ki-67 LI	0.8 (0.705-0.898)	17.5	< 0.001	77.1	73.7
Overall e-Ki-67 LI	0.79 (0.695-0.892)	13.5	< 0.001	74.2	77.1
		D'Amico			

Ki-67 counting method	AUC (95%CI)	Cut-off	p-value	Sensitivity (%)	Specificity (%)
Manual Ki-67 LI	0.75 (0.647-0.851)	11.81	< 0.001	70.7	71
Hot-spot e-Ki-67 LI	0.76 (0.655-0.859)	16	< 0.001	73.2	73
Overall e-Ki-67 LI	0.74 (0.634-0.842)	11	< 0.001	73.2	67

LI**:** labelling index, CAPRA: Cancer of the Prostate Risk Assessment, CI: confidence interval, AUC: Area under the curve

Correlation of Different Ki-67 Counting Methods

We correlated three different Ki-67 counting methods including manual Ki-67 LI, overall e-Ki-67 LI, and hot-spot e-Ki-67 LI. There was a strong positive correlation among all counting methods (**Table 6.3**).

Table 6.3 *Correlation of Ki-67 LI counting methods.*

	r	*p* value
Overall e-Ki-67 LI × Hot-spot Ki-67 LI	0.945	< 0.001
Manual Ki-67 LI × Overall e-Ki-67 LI	0.831	< 0.001
Manual Ki-67 LI × Hot-spot e-Ki-67 LI	0.882	< 0.001

LI: labeling index, r: rank

Correlation of the Ki-67 LI with Clinical, Pathological, and Laboratory Findings

Ki-67 LI calculated by three different counting methods were found to have a significant positive correlation with prognostic factors (GS, GG, cT) and both risk classification systems ($p < 0.001$) (**Table 7.1, 7.2, 7.3).**

Table 7.1. *Correlation of manual Ki-67 LI with prognostic factors and risk classifications.*

Spearman's rho		Manual Ki-67 LI	Total Gleason Score	ISUP Grade	Clinical Stage	D'Amico Risk Group	CAPRA Risk Group
Total Gleason Score	Correlation Coefficient	,56*					
ISUP Grade		,59*	,98*				
Clinical Stage		,42*	,54*	,55*			
D'Amico Risk Group		,48*	,78*	,78*	,60*		
CAPRA Risk Group		,55*	,77*	,78*	,62*	,82*	

* *p < .001*

Table 7.2. *Correlation of overall e-Ki-67 LI with prognostic factors and risk classification systems.*

Spearman's rho		Overall e-Ki-67 LI	Total Gleason Score	ISUP Grade Group	CAPRA Risk Group	D'Amico Risk Group
Total Gleason Score	Correlation Coefficient	,60*				
ISUP Grade Group		,62*	,98*			
Clinical Stage		,45*	,54*	,55*	,62*	,60*
D'Amico Risk Group		,46*	,78*	,78*	,82*	
CAPRA Risk Group		,55*	,77*	,78*		

* $p < .001$

Table 7.3 *Correlation of hot-spot e-Ki-67 LI with prognostic factors and risk classification systems.*

Spearman's rho		Ki-67 LI in Hot Spot Area	Total Gleason Score	ISUP Grade	Clinical Stage	D'Amico Risk Group
Total Gleason Score	Correlation Coefficient	,59*				
ISUP Grade		,61*	,98*			
Clinical Stage		,47*	,54*	,55*		
D'Amico Risk Group		,48*	,78*	,78*	,60*	
CAPRA Risk Group		,55*	,77*	,78*	,62*	,82*

* $p < .001$

Relationship of Ki-67-low and Ki-67-high Groups with Clinical, Pathological, and Laboratory Findings

We found that "Ki-67-low" and "Ki-67-high" groups were significantly associated with prognostic factors (GS, GG, cT) and both risk classification systems ($p < 0.001$) (**Tables 8.1, 8.2, 8.3, 8.4)**

Table 8.1 *Relationship of Ki-67 LI with clinical stages (cT).*

		Manual Ki-67 - 12% cut off			*p* value
		Ki-67-low	Ki-67-high	Total	< 0.001
Clinical stages	cT1*	31 (72.1%)	12 (27.9%)	43	
	cT2	15 (42.9%)	20 (57.1%)	35	
	cT3*	2 (14.3%)	12 (85.7%)	14	
Total		48	44	92	

* Statistically significant associations.

Table 8.2 *Relationship of Ki-67-low and Ki-67-high groups with ISUP grade groups.*

		Manual Ki-67 – 12% cut-off			*p* value
		Ki-67-low n (%)	Ki-67-high n (%)	Total	
ISUP Grade Group	1*	24 (82.75)	5 (17.24)	29	< 0.001
	2	15 (62.5)	9 (37.5)	24	
	3*	3 (25)	9 (75)	12	
	4	4 (33.33)	8 (66.66)	12	
	5*	2 (13.33)	13 (86.66)	15	
Total		48	44	92	

* Statistically significant associations.

Table 8.3 *Relationship of Ki-67-low and Ki-67-high groups with CAPRA risk groups.*

		Manual Ki-67 - 12% cut-off			*p* value
		Ki-67-low, n (%)	Ki-67-high, n (%)	Total	
CAPRA Risk Group	Low Risk	18 (85.71)	3 (14.28)	21	< 0.001
	Intermediate Risk	23 (63.88)	13 (36.11)	36	
	High Risk	7 (20)	28 (80)	35	
Total		48	44	92	

Table 8.4. *Relationship of Ki-67-low and Ki-67-high groups with D'Amico risk groups.*

		Manual Ki-67 - 12% cut-off			*p* value
		Ki-67-low, n (%)	Ki-67-high, n (%)	Total	
D'Amico Risk Group	Low Risk	17 (85)	3 (15)	20	< 0.001
	Intermediate Risk	19 (61.29)	12 (38.7)	31	
	High Risk	12 (29.26)	29 (70.73)	41	
Total		48	44	92	

We did not find a difference in terms of homogeneity in staining between low- and high-grade PCA cases (p = 0,108) (**Table 9.1**).

Table 9.1. *Relationship of homogeneity in staining with low-grade and high-grade prostate cancers.*

			Prostate Cancer Grade		
			Low grade	High grade	Total
Homogeneity	Homogenous	n	33	19	52
		%	50,8%	70,4%	56,5%
	Heterogenous	n	32	8	40
		%	49,2%	29,6%	43,5%
Total		n	65	27	92
		%	100%	100%	100%

There was no difference in terms of homogeneity in staining between Ki-67-low and -high groups (p = 0.714) (**Table 9.2**).

Table 9.2. *Relationship of homogeneity in staining with Ki-67-low and Ki-67-high groups.*

		Manual Ki-67 – 12% cut-off			p value
		Low Ki-67	High Ki-67	Total	
Homogeneity	Homogenous	28 (53.85%)	24 (46.15%)	52	0.714
	Heterogenous	20 (50%)	20 (50%)	40	
Total		48	44	92	

There was no statistically significant association between homogeneity in staining of Ki-67 and CAPRA and D'Amico risk classifications (p = 0.065, p = 0.081, respectively) (**Tables 9.3, 9.4**).

Table 9.3. *Relationship of homogeneity of Ki-67 staining with CAPRA risk groups.*

		CAPRA Risk Group				p value
		Low Risk	Intermediate Risk	High Risk	Total	
Homogeneity	Homogenous	11 (21.15%)	16 (30.76%)	25 (48.07%)	52	0.065
	Heterogenous	10 (25%)	20 (50%)	10 (25%)	40	
Total		21	36	35	92	

Table 9.4. *Relationship of homogeneity of Ki-67 staining with D'Amico risk groups.*

		D'Amico Risk Group				p value
		Low Risk	Intermediate Risk	High Risk	Total	
Homogeneity	Homogenous	11 (21.15%)	13 (25%)	28 (53.85%)	52	0.081
	Heterogenous	9 (22.5%)	18 (45%)	13 (32.5%)	40	
Total		20	31	41	92	

The low and intermediate PCA risk groups were combined as a low-risk group and binary risk groups (low/high) were created. Ki-67 LI was also found to be significantly associated with binary CAPRA and D'Amico risk classifications ($p < 0.001$) (**Tables 10.1, 10.2**).

Table 10.1. *Relationship of manual Ki-67 LI with binary CAPRA risk groups.*

		Manual Ki-67 - 12% cut-off			*p* value
		Low Ki-67	High Ki-67	Total	
CAPRA Risk Group	Low Risk	41 (71.92%)	16 (28.07%)	57	< 0.001
	High Risk	7 (20%)	28 (80%)	35	
Total		48	44	92	

Table 10.2. *Relationship of manual Ki-67 LI with binary D'Amico risk groups.*

		Manual Ki-67 - 12% cut off			*p* value
		Low Ki-67	High Ki-67	Total	
D'Amico Risk Group	Low Risk	36 (70.59%)	15 (29.41%)	51	< 0.001
	High Risk	12 (29.26%)	29 (70.73%)	41	
Total		48	44	92	

Supplementary Figures:

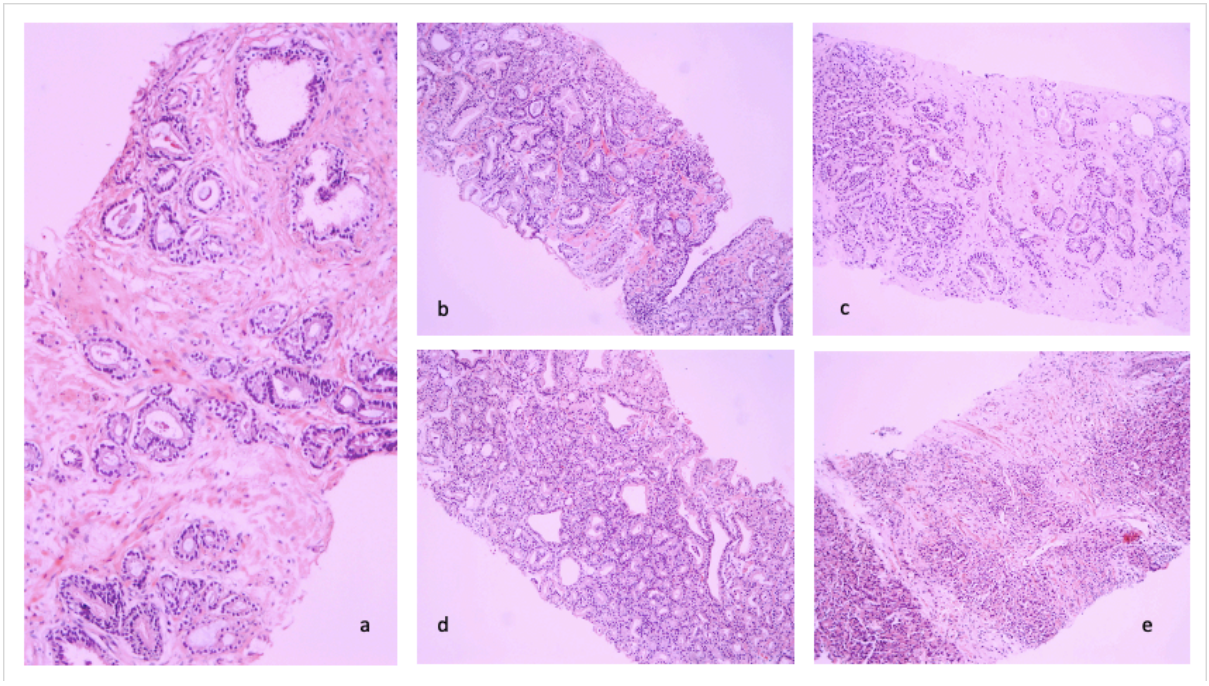

Figure S1. ISUP grade groups (GG) in acinar adenocarcinoma of the prostate. **a**. ISUP GG 1 (3+3), **b**. ISUP GG 2 (3+4), **c**. ISUP GG 3 (4+3), **d**. ISUP GG 4 (4+4), **e**. ISUP GG 5 (5+5) (H&E stain, x100 magnification)

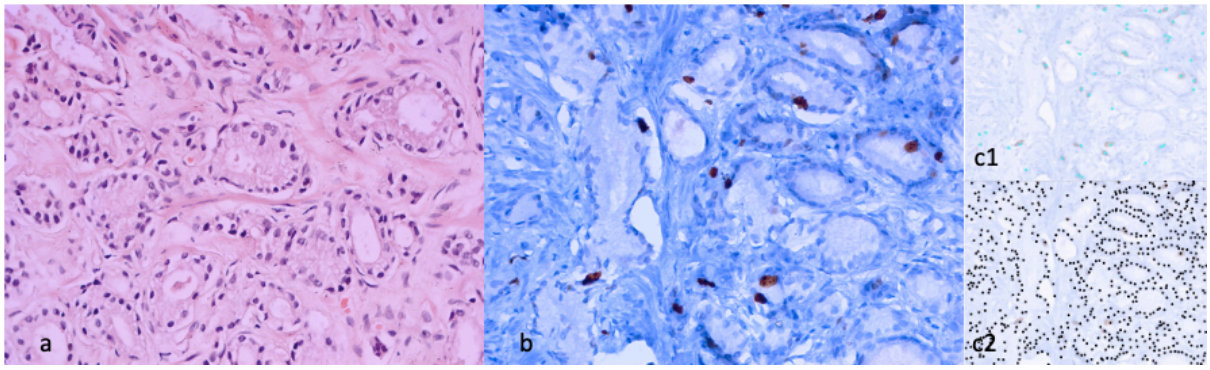

Figure S2. Example of an adenocarcinoma of the prostate with low Ki-67 labeling index (LI). **a**. This image shows a part of an H&E-stained slide, **b**. Ki-67 LI is 6.38%, **c1.** Counting Ki-67 positive cells (blue dots) with the help of Ai-assisted mobile software, **c2.** Counting all tumor cells (black dots). (x400 magnification)

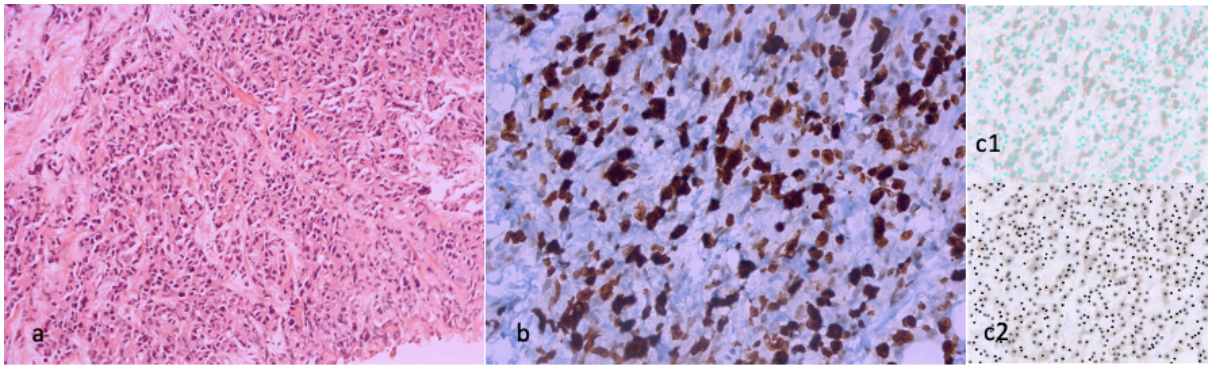

Figure S3. Example of an adenocarcinoma of the prostate with high Ki-67 labeling index (LI). **a**. This image shows a part of an H&E-stained slide, **b**. Ki-67 LI is 81.07%, **c1.** Counting Ki-67 positive cells (blue dots) with the help of Ai-assisted mobile software, **c2.** Counting all tumor cells (black dots). (x400 magnification)

CHAPTER V
Discussion

Ki-67 LI has long been used in pathology practice in distinguishing neoplastic lesions from nonneoplastic lesions, e.g., atrophy vs dysplasia of the uterine cervix, grading of tumors, e.g. the lung and gastrointestinal neuroendocrine tumors, and in prognostication, e.g., breast carcinomas. In this study, we aimed to understand the clinicopathological importance of Ki-67 LI in PCA, particularly in robust clinicopathological risk classification systems including D'Amico and CAPRA. Using three different Ki-67 LI counting techniques, we showed a strong positive correlation between Ki-67 LI and both risk classifiers. The cut-off value determined as 12% was also able to stratify PCA cases into low- and high-risk groups, efficiently. Our results support that Ki-67 can be used as a supportive or surrogate tool to determine the risk status of PCA cases and in choosing a more personalized treatment approach.

Prostate cancer is a disease of men mostly over 65 years of age, and the risk of developing PCA increases in direct proportion to increasing age (Rawla 2019). In our series, the age of the patients ranged from 44 to 87 (mean 69.82 years). One of the screening tools for PCA is PSA levels in the blood. It was shown that the PSA levels are often more than 2.5 ng/mL in PCA patients, however, lower levels do not guarantee excluding the presence of cancer (Thompson et al., 2004). In addition, diagnosis of PCA is over 50% in patients with PSA levels of more than 10. In our cohort, PSA levels ranged from 0.87 to 3625 (median 9,7950). Among 92 cases, 86 (93.5%) had PSA levels higher than 4 ng/mL, and 43 (46.7%) had higher than 10.

When a patient is suspected of PCA, a core needle biopsy procedure is indicated. The biopsy samples are examined histopathologically in diagnosis, grading, and staging. If a diagnosis of PCA is made, a GG (1 to 5) is assigned based on ISUP 2014 system (Epstein, Egevad, et al., 2016). GGs correlate with the biological behaviour of cancer. The higher the GG, the more aggressive cancer. Most cases are detected in lower-GGs as in our series (Epstein, Egevad, et al., 2016). From biopsy samples, the calculation of the percentage of positive biopsy cores provides outcome prediction as is used in clinical risk classification systems, such as in CAPRA (M. Cooperberg et al., 2005). For staging, the extent of the tumor (T) is determined clinically (cT) if biopsy samples

are received or pathologically (pT) if radical resection is done. We assigned a cT in our cases based on the results of DRE, MRI, and prostate biopsy (Hoedemaeker et al., 2000). Most PCA patients were detected at an early stage at the time of diagnosis in our series compatible with the previous studies (Brawley, 2012; M. R. Cooperberg, Moul, et al., 2005).

There has been accumulated evidence on the prognostic significance of Ki-67 LI in PCA. A meta-analysis of 21 selected studies between 1996 and 2014 comprising 5419 patients showed that Ki-67 LI is significantly associated with biochemical failure-free survival, disease-free survival, disease-specific survival, distant metastasis rate, and overall survival (Berlin et al., 2017a). Following this, in a multi-institutional study on 1004 radical prostatectomy, Ki-67 LI was found to be associated with stage ($p < 0.001$), seminal vesicle invasion (SVI, $p = 0.02$), extracapsular extension ($p < 0.001$), and GS ($p < 0.001$) (Tretiakova et al., 2016a). A 12% higher risk of dying from cancer was linked to every 1% rise in Ki-67 expression after adjusting for perineural invasion and GS ($p < 0.001$). Along with GS and the presence of perineural invasion, using Ki-67 was suggested in predicting long-term outcomes of PCA patients (Tollefson et al., 2014). High Ki-67 LI was also found to be associated with positive margins ($p = 0.001$), extra-capsular extension ($p < 0.001$), and greater tumor size ($\geq$ 20 mm, $p = 0.03$) in two studies (Fantony et al., 2018a; Richardsen et al., 2017a). In a recent study, Ki-67 mRNA levels were assessed in 492 PCA cases compared to 52 normal samples using RNA-seq data from The Cancer Genome Atlas PC datasets. Ki-67 upregulation was found to be associated with cancer tissue ($p < 0.001$) and worst disease-free survival ($p = 0.035$). Immunohistochemical studies on 94 biopsies showed that Ki-67 was associated with the increase in the ISUP score ($p < 0.001$), cancer stage ($p = 0.05$), biochemical recurrence ($p = 0.0006$), and metastasis ($p < 0.001$). A positive correlation was reported between Ki-67 expression and ISUP score ($r = 0.5112$, $p < 0.001$) and disease risk stratification ($r = 0.3388$, $p = 0.0009$) (Maia, Dos Santos, et al., 2022). Three studies on low, intermediate, and high-risk PCA patients treated with radiation with or without androgen deprivation conducted by the Radiation Therapy Oncology Group showed an independent prognostic value of Ki-67 LI. The authors suggested using Ki-67 LI as a stratification factor in future trials (R. Li et al., 2004; Pollack et al., 2004; Verhoven et al., 2013).

A few classification systems have been used to assess PCA risk, including the D'Amico, CAPRA, and AUA/ASTRO/SUO. The D'Amico risk classification system is based on the cT, PSA level, and GS (A. V. D'Amico et al., 1998; Hernandez et al., 2007a). In 2005, CAPRA risk assessment was developed by the UCSF to enhance the precision of the D'Amico classification. It is determined by pathological factors from biopsy samples and several clinical factors including the age, PSA level, GS, cT, and percentage of positive biopsy cores (M. Cooperberg et al., 2005; Lughezzani et al., 2010). Two studies have evaluated the relationship of Ki-67 to risk classifications. In one of these studies, high Ki-67 was associated with disease recurrence (HR = 9.20, 95% CI: 1.27–66.44) and progression (HR = 2.97, 95% CI: 1.05–8.43) in patients with low/intermediate risk CAPRA score (Lobo et al., 2018). The other study used a modified version of D'Amico (according to AUA/ASTRO/SUO guideline) with parameters including PSA level, PSA density, GG, cT, biopsy core positivity, and percentage of positive core (Maia, Dos Santos, et al., 2022; Sanda et al., 2018a). They found a positive correlation between Ki-67 expression and disease risk stratification ($r = 0.3388$, $p = 0.0009$). In our study, we assessed the relationship of Ki-67 LI with each of D'Amico and CAPRA risk classifications. We found a significant positive correlation between Ki-67 LI and both classifications ($p < 0.001$). In addition, the cut-off value, determined as 12% based on ROC curve analyses, was able to divide most of the cases into high risk and low risk ($p < 0.001$). Our results supported the use of Ki-67 LI in determining the clinical risk of PCA patients.

Prostate cancer is a complex and biologically heterogeneous disease. Therefore, novel predicting markers have been researched to distinguish low-risk individuals with indolent tumors to prevent overtreatment (Richardsen et al., 2017a). A few studies found Ki-67 LI prognostic in low-risk PCA patients. Ki-67 as a continuous variable was shown to be a significant predictor of time to death from PCA in GGs 1 and 2 (Kammerer-Jacquet et al., 2019a). In addition, Ki-67 LI was found to be an independent prognostic factor in case of a low total percentage of biopsy tissue with tumor (< 7%) or low GS (< 7) (the hazard ratio being 6.76 and 6.44, respectively) (Zellweger et al., 2009). Another study showed that high Ki-67 LI was associated with a worse prognosis in patients with low/intermediate risk CAPRA score, as previously mentioned (Lobo et al., 2018). These results suggest that high Ki-67 may assist in revealing more aggressive cases among low-risk PCA patients. One recent study

investigated Ki-67 LI in 112 PCA cases with high GSs (≥8) (Vlajnic et al., 2022). Fifteen cases showed low (≤10% staining) Ki-67 LI. The clinical significance of this finding is yet to be elucidated.

There is a limited number of studies on the predictive value of Ki-67 LI. In phase 3, multicentre, randomized controlled trial called "Conventional or hypo fractionated High Dose Intensity Modulated Radiotherapy for Prostate Cancer (CHHIP)", it was studied how different radiation therapy fractionation schedules affected Ki-67 LI in localized PCA. It is hypothesized that the cancers with high proliferative rates would be insensitive to fraction size and be prone to recur after the reduced total dose in hypo fractionated (>2 Gy) schedules. In contrast, it was anticipated that tumors with modest rates of proliferative growth would be sensitive to fraction size and so more prone to relapse following traditional fractionation (2 Gy) schedules. They did not find any significant relationship between Ki-67 and the fractionation schedule (Wilkins et al., 2018). In a trial conducted by the Radiation Therapy Oncology Group, several biomarkers including Ki-67 were analysed to find out who benefitted from short- versus long-term hormone therapy. They found no evidence of statistically significant interactions between biomarkers and treatment (short-term androgen deprivation therapy vs. long-term androgen deprivation therapy). However, when they checked the markers individually, they found the effects of Ki-67 were larger in patients receiving long-term androgen deprivation therapy (Pollack et al., 2014). The predictive value of Ki-67 LI in PCA should be further studied in different patient cohorts and treatment modalities.

Different cut-off values have been selected, ranging from 5% to 10% in most of the studies summarized in (Kammerer-Jacquet et al., 2019b). The variability in the cut-off values can be affected by the characteristics of the cohort and clinical endpoints. Various studies have been conducted on either radical prostatectomy, core biopsy, or transurethral resection specimens. The prognostic value has been evaluated by the clinical endpoints including cancer-specific survival and/or metastasis-free survival, or other powerful prognostic factors including the stage, GG, PSA level, and total percentage of biopsy tissue with tumor. For more personalized patient management, risk classification systems were developed including several of these most important prognostic factors. In our study, the cut-off value was determined in core biopsy

samples based on robust clinical risk classification systems including D'Amico and CAPRA.

Methodology (i.e. counting method) is another significant factor affecting the cut-off value. Overall, four different Ki-67 counting and scoring methods have been suggested: eye-balling estimation, visual counting using a microscope or viewer software, manual counting of camera-captured or digital images, and using an automated counting system (Dzulkifli et al., 2018). These methods have certain advantages and disadvantages over each other. The first two methods do not require an additional cost, however, there is poor reproducibility. Automatic counting provides saving time and reproducibility; however, it is costly and has moderate accuracy due to overcounting unwanted cells and objects and is affected by staining quality. Manual counting is a reliable technique with high accuracy. This method can be time-consuming and necessitates a camera capable of capturing images. However, the cameras are relatively inexpensive and widely used in pathology laboratories. We have used and compared three practical methods: overall e-Ki-67 LI (eye-balling in the entire tumor area in a biopsy), hot-spot e-Ki-67 LI (eye-balling in the hot-spot area), and manual Ki-67 LI (manual counting in the hot-spot area). We found strong correlation among these methods ($p < 0{,}001$). All methods were found to be significantly correlated with PCA risk classifications ($p < 0{,}001$). In addition, the defined cut-off values according to each of the D'Amico and CAPRA risk classification systems were found to be very close (**Tables 6.1 and 6.2**). These results showed that any of these methods may provide powerful information about the clinical risk of PCA patients. Nevertheless, our method of choice in our clinicopathological analyses was manual counting (manual Ki-67 LI) for two reasons: i) in terms of high accuracy and reproducibility in assessment ii) hot-spot tumor areas may better represent the tumor biology (Dzulkifli et al., 2018). Instead of counting cells on printed images, we used free mobile software (CFU.Ai v1.4) allowing to click on and select or deselect the cells one by one. In addition, the software provides Ai assistance indicating Ki-67 positive cells which may be manually deselected when needed. Once you finish counting, it automatically gives the total number of selected cells and the images can be saved and stored with the results easily. Using this method, we found 12% as the optimal cut-off value for Ki-67 to determine the PCA risk group.

Next-generation sequencing revealed considerable variability in genomic alterations in localized PCAs (Wei et al., 2017). Tumor heterogeneity may result in variability in staining which has been reported as a possible confounding factor in Ki-67 interpretation. In one study on high-grade PCA cases (GG 4 and 5), 41% with high Ki-67 LI (>10%) had areas of low and high Ki-67 LI reflecting intratumoral heterogeneity. The morphologic homogeneity observed in a group of these tumors suggested the presence of molecular heterogeneity (Vlajnic et al., 2022). Aiming to highlight a part of the molecular basis of this intratumoral variability of Ki-67, the authors tested two commonly altered markers in PCA including Bcl-2 and PTEN. Although they could not find any association, the molecular complexity of cancer was emphasized for further marker analysis.

There have been some drawbacks in our study including a relatively low number of cases and lack of follow-up information. However, our data was sufficient to perform PCA risk classifications which were proven to be powerful in prognostic assessment. Manual counting of Ki-67 staining is relatively more time-consuming. On the other hand, Ai-assisted mobile software has made it much simpler, more accurate, and more accessible. Intratumoral heterogeneity should be addressed among the factors which may confound the results of Ki-67 LI in certain cases. This issue might be tested and overcome in future studies by performing the stain on multiple core needle biopsies. Nevertheless, to date, there is no data regarding the negative effects of heterogeneity on the prognostic value of Ki-67 in PCA.

CHAPTER VI

Conclusion

Previous studies have shown the prognostic importance of Ki-67 LI in PCA, although there was limited information about the relationship between Ki-67 LI and PCA risk classifications. In this study, our primary aim was to assess whether there was a relationship between Ki-67 LI and robust PCA risk classification systems including D'Amico and CAPRA. For this purpose, we used the manual Ki-67 counting method, which is a highly objective, accurate, and reproducible technique. We showed that there is a strong positive correlation between Ki-67 LI and both risk classifications ($p < 0.001$). In addition, our cut-off value (12%) was able to divide most of the cases into high-risk and low-risk ($p < 0.001$). Using two other Ki-67 counting methods, hot-spot e-Ki-67 LI and overall e-Ki-67 LI, did not affect this powerful association ($p < 0.001$). We also showed that Ki-67 LI has a significant association with important prognostic factors in PCA such as stage, GS, and GG. Our results support that Ki-67 can be used as a supportive or surrogate tool to determine the risk status of PCA cases and in choosing a more personalized treatment approach.

Recommendations

- Ki-67 can be used as a powerful tool in determining the risk of PCA patients.
- Ki-67 can be used as a strong prognostic biomarker in PCA patients.
- We suggest using the manual Ki-67 counting method for objective, accurate, and reproducible results.
- Further studies are needed to determine the value of high Ki-67 LI in low-risk patients and low Ki-67 LI in high-risk patients.
- Ki-67 LI may be used to determine which patients in the low-risk group should undergo active surveillance or which patients in the high-risk group with localized disease should receive adjuvant therapy. Large prospective studies are suggested to highlight these points.
- In cases when clinical management is indeterminate, the urologists may request a Ki-67 test to support their decision on whether to follow up or treat the patient according to the result.

REFERENCES

Berlin, A., Castro-Mesta, J. F., Rodriguez-Romo, L., Hernandez-Barajas, D., González-Guerrero, J. F., Rodríguez-Fernández, I. A., González-Conchas, G., Verdines-Perez, A., & Vera-Badillo, F. E. (2017). Prognostic role of Ki-67 score in localized prostate cancer: A systematic review and meta-analysis. *Urologic Oncology: Seminars and Original Investigations*, *35*(8), 499–506. https://doi.org/10.1016/j.urolonc.2017.05.004

Cooperberg, m. R., pasta, d. J., elkin, e. P., litwin, m. S., latini, d. M., du chane, j., & carroll, p. R. (2005). The university of california, san francisco cancer of the prostate risk assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. *Journal of Urology*, *173*(6), 1938–1942. https://doi.org/10.1097/01.ju.0000158155.33890.e7

D'Amico, G., & Luca, A. (1997). 3 Natural history. Clinical-haemodynamic correlations. Prediction of the risk of bleeding. *Baillière's Clinical Gastroenterology*, *11*(2), 243–256. https://doi.org/10.1016/S0950-3528(97)90038-5

Fantony, J. J., Howard, L. E., Csizmadi, I., Armstrong, A. J., Lark, A. L., Galet, C., Aronson, W. J., & Freedland, S. J. (2018). Is Ki67 prognostic for aggressive prostate cancer? A multicenter real-world study. *Biomarkers in Medicine*, *12*(7), 727–736. https://doi.org/10.2217/bmm-2017-0322

Hirabayashi, K., Zamboni, G., Nishi, T., Tanaka, A., Kajiwara, H., & Nakamura, N. (2013). Histopathology of gastrointestinal neuroendocrine neoplasms. *Frontiers in Oncology*, *3*, 2. https://doi.org/10.3389/fonc.2013.00002

Humphrey, P. A. (2017). Histopathology of Prostate Cancer. *Cold Spring Harbor Perspectives in Medicine*, *7*(10), a030411. https://doi.org/10.1101/cshperspect.a030411

Kamal, M. (2022). Cervical Pre-cancers: Biopsy and Immunohistochemistry. *Cytojournal*, *19*, 38. https://doi.org/10.25259/CMAS_03_13_2021

Li, l. T., jiang, g., chen, q., & zheng, j. N. (2015). Ki67 is a promising molecular target in the diagnosis of cancer (Review). *Molecular Medicine Reports*, *11*(3), 1566–1572. https://doi.org/10.3892/mmr.2014.2914

Maia, r., santos, g. A. Dos, reis, s., viana, n. I., pimenta, r., guimarães, v. R., recuero, s., romão, p., leite, k. R. M., srougi, m., & passerotti, c. C. (2022). Can we use Ki67 expression to predict prostate cancer aggressiveness? *Revista Do Colégio Brasileiro de Cirurgiões*, *49*. https://doi.org/10.1590/0100-6991e-20223200-en

Nielsen, T. O., Leung, S. C. Y., Rimm, D. L., Dodson, A., Acs, B., Badve, S., Denkert, C., Ellis, M. J., Fineberg, S., Flowers, M., Kreipe, H. H., Laenkholm, A.-V., Pan, H., Penault-Llorca, F. M., Polley, M.-Y., Salgado, R., Smith, I. E., Sugie, T., Bartlett, J. M. S., … Hayes, D. F.

(2021). Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. *JNCI: Journal of the National Cancer Institute*, *113*(7), 808–819. https://doi.org/10.1093/jnci/djaa201

Pelosi, G., Rindi, G., Travis, W. D., & Papotti, M. (2014). Ki-67 Antigen in Lung Neuroendocrine Tumors: Unraveling a Role in Clinical Practice. *Journal of Thoracic Oncology*, *9*(3), 273–284. https://doi.org/10.1097/JTO.0000000000000092

Rawla, P. (2019). Epidemiology of Prostate Cancer. *World Journal of Oncology*, *10*(2), 63–89. https://doi.org/10.14740/wjon1191

Richardsen, E., Andersen, S., Al-Saad, S., Rakaee, M., Nordby, Y., Pedersen, M. I., Ness, N., Grindstad, T., Movik, I., Dønnem, T., Bremnes, R., & Busund, L.-T. (2017). Evaluation of the proliferation marker Ki-67 in a large prostatectomy cohort. *PLOS ONE*, *12*(11), e0186852. https://doi.org/10.1371/journal.pone.0186852

Scholzen, T., & Gerdes, J. (2000). The Ki-67 protein: From the known and the unknown. *Journal of Cellular Physiology*, *182*(3), 311–322. https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

Tretiakova, M. S., Wei, W., Boyer, H. D., Newcomb, L. F., Hawley, S., Auman, H., Vakar-Lopez, F., McKenney, J. K., Fazli, L., Simko, J., Troyer, D. A., Hurtado-Coll, A., Thompson, I. M., Carroll, P. R., Ellis, W. J., Gleave, M. E., Nelson, P. S., Lin, D. W., True, L. D., … Brooks, J. D. (2016). Prognostic value of Ki67 in localized prostate carcinoma: a multi-institutional study of >1000 prostatectomies. *Prostate Cancer and Prostatic Diseases*, *19*(3), 264–270. https://doi.org/10.1038/pcan.2016.12

Wang, L., Lu, B., He, M., Wang, Y., Wang, Z., & Du, L. (2022). Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. *Frontiers in Public Health*, *10*, 176. https://doi.org/10.3389/fpubh.2022.811044

Al-Ubaidi, F. L. T., Schultz, N., Egevad, L., Granfors, T., & Helleday, T. (2012). Castration Therapy of Prostate Cancer Results in Downregulation of HIF-1α Levels. *International Journal of Radiation Oncology*Biology*Physics*, *82*(3), 1243–1248. https://doi.org/10.1016/j.ijrobp.2011.10.038

Andrews, C., & Humphrey, P. A. (2014). Utility of ERG Versus AMACR Expression in Diagnosis of Minimal Adenocarcinoma of the Prostate in Needle Biopsy Tissue. *American Journal of Surgical Pathology*, *38*(7), 1007–1012. https://doi.org/10.1097/PAS.0000000000000205

Aus, G., Damber, J.-E., Khatami, A., Lilja, H., Stranne, J., & Hugosson, J. (2005). Individualized Screening Interval for Prostate Cancer Based on Prostate-Specific Antigen Level. *Archives of Internal Medicine*, *165*(16), 1857. https://doi.org/10.1001/archinte.165.16.1857

Baig, F. A., Hamid, A., Mirza, T., & Syed, S. (2015). Ductal and Acinar Adenocarcinoma of Prostate: Morphological and Immunohistochemical Characterization. *Oman Medical Journal*, *30*(3), 162–166. https://doi.org/10.5001/omj.2015.36

Barakzai, M. A. (2019). Prostatic Adenocarcinoma: A Grading from Gleason to the New Grade-Group System: A Historical and Critical Review. *Asian Pacific Journal of Cancer Prevention*, *20*(3), 661–666. https://doi.org/10.31557/APJCP.2019.20.3.661

Beheshti, M., Schöder, H., Walz, J., Rezaee, A., & Langsteger, W. (2018). Prostate Cancer. In *PET/CT in Cancer: An Interdisciplinary Approach to Individualized Imaging* (pp. 199–219). Elsevier. https://doi.org/10.1016/B978-0-323-48567-8.00010-9

Boorjian, S. A., Karnes, R. J., Rangel, L. J., Bergstralh, E. J., & Blute, M. L. (2008). Mayo Clinic Validation of the D'Amico Risk Group Classification for Predicting Survival Following Radical Prostatectomy. *Journal of Urology*, *179*(4), 1354–1361. https://doi.org/10.1016/j.juro.2007.11.061

Bratt, O., & Lilja, H. (2015). Serum markers in prostate cancer detection. *Current Opinion in Urology*, *25*(1), 59–64. https://doi.org/10.1097/MOU.0000000000000128

Broyde, A., Boycov, O., Strenov, Y., Okon, E., Shpilberg, O., & Bairey, O. (2009). Role and prognostic significance of the Ki-67 index in non-Hodgkin's lymphoma. *American Journal of Hematology*, *84*(6), 338–343. https://doi.org/10.1002/ajh.21406

Catalona, W. J., Partin, A. W., Slawin, K. M., Brawer, M. K., Flanigan, R. C., Patel, A., Richie, J. P., deKernion, J. B., Walsh, P. C., Scardino, P. T., Lange, P. H., Subong, E. N. P., Parson, R. E., Gasior, G. H., Loveland, K. G., & Southwick, P. C. (1998). Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease. *JAMA*, *279*(19), 1542. https://doi.org/10.1001/jama.279.19.1542

Cooperberg, M. R., Hilton, J. F., & Carroll, P. R. (2011). The CAPRA-S score. *Cancer*, *117*(22), 5039–5046. https://doi.org/10.1002/cncr.26169

Cuylen, S., Blaukopf, C., Politi, A. Z., Müller-Reichert, T., Neumann, B., Poser, I., Ellenberg, J., Hyman, A. A., & Gerlich, D. W. (2016). Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. *Nature*, *535*(7611), 308–312. https://doi.org/10.1038/nature18610

D'Amico, G., & Luca, A. (1997). 3 Natural history. Clinical-haemodynamic correlations. Prediction of the risk of bleeding. *Baillière's Clinical Gastroenterology*, *11*(2), 243–256. https://doi.org/10.1016/S0950-3528(97)90038-5

Deep, G., Kumar, R., Nambiar, D. K., Jain, A. K., Ramteke, A. M., Serkova, N. J., Agarwal, C., & Agarwal, R. (2017). Silibinin inhibits hypoxia-induced HIF-1α-mediated signaling, angiogenesis and lipogenesis in prostate cancer cells: In vitro evidence and in vivo functional imaging and metabolomics. *Molecular Carcinogenesis*, *56*(3), 833–848. https://doi.org/10.1002/mc.22537

Descotes, J.-L. (2019). Diagnosis of prostate cancer. *Asian Journal of Urology*, *6*(2), 129–136. https://doi.org/10.1016/j.ajur.2018.11.007

Dyba, T., Randi, G., Bray, F., Martos, C., Giusti, F., Nicholson, N., Gavin, A., Flego, M., Neamtiu, L., Dimitrova, N., Negrão Carvalho, R., Ferlay, J., & Bettio, M. (2021). The European cancer burden in 2020: Incidence and mortality estimates for 40 countries and 25 major

cancers. *European Journal of Cancer*, *157*, 308–347. https://doi.org/10.1016/j.ejca.2021.07.039

Epstein, J. I., Amin, M. B., Reuter, V. E., & Humphrey, P. A. (2017). Contemporary Gleason Grading of Prostatic Carcinoma. *American Journal of Surgical Pathology*, *41*(4), e1–e7. https://doi.org/10.1097/PAS.0000000000000820

Epstein, J. I., Egevad, L., Humphrey, P. A., & Montironi, R. (2014). Best Practices Recommendations in the Application of Immunohistochemistry in the Prostate. *American Journal of Surgical Pathology*, *38*(8), e6–e19. https://doi.org/10.1097/PAS.0000000000000238

Epstein, J. I., Zelefsky, M. J., Sjoberg, D. D., Nelson, J. B., Egevad, L., Magi-Galluzzi, C., Vickers, A. J., Parwani, A. v., Reuter, V. E., Fine, S. W., Eastham, J. A., Wiklund, P., Han, M., Reddy, C. A., Ciezki, J. P., Nyberg, T., & Klein, E. A. (2016). A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *European Urology*, *69*(3), 428–435. https://doi.org/10.1016/j.eururo.2015.06.046

Esgueva, R., Perner, S., J LaFargue, C., Scheble, V., Stephan, C., Lein, M., Fritzsche, F. R., Dietel, M., Kristiansen, G., & Rubin, M. A. (2010). Prevalence of TMPRSS2–ERG and SLC45A3–ERG gene fusions in a large prostatectomy cohort. *Modern Pathology*, *23*(4), 539–546. https://doi.org/10.1038/modpathol.2009.193

Fraga, A., Ribeiro, R., Príncipe, P., Lopes, C., & Medeiros, R. (2015). Hypoxia and Prostate Cancer Aggressiveness: A Tale With Many Endings. *Clinical Genitourinary Cancer*, *13*(4), 295–301. https://doi.org/10.1016/j.clgc.2015.03.006

Gadbail, a. R., sarode, s. C., chaudhary, m. S., gondivkar, s. M., tekade, s. A., yuwanati, m., & patil, s. (2021). Ki67 Labelling Index predicts clinical outcome and survival in oral squamous cell carcinoma. *Journal of Applied Oral Science*, *29*. https://doi.org/10.1590/1678-7757-2020-0751

Goetze, S., Schüffler, P., Athanasiou, A., Koetemann, A., Poyet, C., Fankhauser, C. D., Wild, P. J., Schiess, R., & Wollscheid, B. (2022). Use of MS-GUIDE for identification of protein biomarkers for risk stratification of patients with prostate cancer. *Clinical Proteomics*, *19*(1), 9. https://doi.org/10.1186/s12014-022-09349-x

Greenberg, J. B., McIntosh, A. C., & Brindley, J. (2001). Linear stability analysis of laminar premixed spray flames. *Proceedings of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences*, *457*(2005), 1–31. https://doi.org/10.1098/rspa.2000.0653

Grubb, R. L., Pinsky, P. F., Greenlee, R. T., Izmirlian, G., Miller, A. B., Hickey, T. P., Riley, T. L., Mabie, J. E., Levin, D. L., Chia, D., Kramer, B. S., Reding, D. J., Church, T. R., Yokochi, L. A., Kvale, P. A., Weissfeld, J. L., Urban, D. A., Buys, S. S., Gelmann, E. P., … Andriole, G. L. (2008). Prostate cancer screening in the Prostate, Lung, Colorectal and Ovarian cancer screening trial: update on findings from the initial four rounds of screening in a randomized

trial. *BJU International*, *102*(11), 1524–1530. https://doi.org/10.1111/j.1464-410X.2008.08214.x

Han, C., Zhu, L., Liu, X., Ma, S., Liu, Y., & Wang, X. (2021). Differential diagnosis of uncommon prostate diseases: combining mpMRI and clinical information. *Insights into Imaging*, *12*(1), 79. https://doi.org/10.1186/s13244-021-01024-3

Hernandez, D. J., Nielsen, M. E., Han, M., & Partin, A. W. (2007). Contemporary Evaluation of the D'Amico Risk Classification of Prostate Cancer. *Urology*, *70*(5), 931–935. https://doi.org/10.1016/j.urology.2007.08.055

Hessels, D., Klein Gunnewiek, J. M. T., van Oort, I., Karthaus, H. F. M., van Leenders, G. J. L., van Balken, B., Kiemeney, L. A., Witjes, J. A., & Schalken, J. A. (2003). DD3PCA3-based Molecular Urine Analysis for the Diagnosis of Prostate Cancer. *European Urology*, *44*(1), 8–16. https://doi.org/10.1016/S0302-2838(03)00201-X

Holger Moch, P. A. H. T. M. U. V. E. R. (Ed.). (2016). *WHO Classification of Tumours of the Urinary System and Male Genital Organs*. International Agency for Research on Cancer (IARC) 69372 Lyon Cedex 08, France.

Huang, H., Guma, S. R., Melamed, J., Zhou, M., Lee, P., & Deng, F.-M. (2018). NKX3.1 and PSMA are sensitive diagnostic markers for prostatic carcinoma in bone metastasis after decalcification of specimens. *American Journal of Clinical and Experimental Urology*, *6*(5), 182–188. http://www.ncbi.nlm.nih.gov/pubmed/30510970

Humphrey, P. A. (2017). Histopathology of Prostate Cancer. *Cold Spring Harbor Perspectives in Medicine*, *7*(10), a030411. https://doi.org/10.1101/cshperspect.a030411

Humphrey, P. A., Moch, H., Cubilla, A. L., Ulbright, T. M., & Reuter, V. E. (2016). The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs—Part B: Prostate and Bladder Tumours. *European Urology*, *70*(1), 106–119. https://doi.org/10.1016/j.eururo.2016.02.028

Jamaspishvili, T., Berman, D. M., Ross, A. E., Scher, H. I., de Marzo, A. M., Squire, J. A., & Lotan, T. L. (2018). Clinical implications of PTEN loss in prostate cancer. *Nature Reviews Urology*, *15*(4), 222–234. https://doi.org/10.1038/nrurol.2018.9

Kim, S. J., Kim, B. S., Choi, C. W., Choi, J., Kim, I., Lee, Y.-H., & Kim, J. S. (2007). Ki-67 expression is predictive of prognosis in patients with stage I/II extranodal NK/T-cell lymphoma, nasal type. *Annals of Oncology*, *18*(8), 1382–1387. https://doi.org/10.1093/annonc/mdm183

Klöppel, G., & la Rosa, S. (2018). Ki67 labeling index: assessment and prognostic role in gastroenteropancreatic neuroendocrine neoplasms. *Virchows Archiv*, *472*(3), 341–349. https://doi.org/10.1007/s00428-017-2258-0

Lee, S.-O., Kim, J.-S., Lee, M.-S., & Lee, H.-J. (2016). Anti-cancer effect of pristimerin by inhibition of HIF-1α involves the SPHK-1 pathway in hypoxic prostate cancer cells. *BMC Cancer*, *16*(1), 701. https://doi.org/10.1186/s12885-016-2730-2

Li, l. T., jiang, g., chen, q., & zheng, j. N. (2015). Ki67 is a promising molecular target in the diagnosis of cancer (Review). *Molecular Medicine Reports*, *11*(3), 1566–1572. https://doi.org/10.3892/mmr.2014.2914

Loeb, S., & Catalona, W. J. (2007). Prostate-specific antigen in clinical practice. *Cancer Letters*, *249*(1), 30–39. https://doi.org/10.1016/j.canlet.2006.12.022

Lopez-Beltran, A., Kirkali, Z., Montironi, R., Blanca, A., Algaba, F., Scarpelli, M., Yorukoglu, K., Hartmann, A., & Cheng, L. (2012). Unclassified renal cell carcinoma: a report of 56 cases. *BJU International*, *110*(6), 786–793. https://doi.org/10.1111/j.1464-410X.2012.10934.x

Lotan, T. L., Tomlins, S. A., Bismar, T. A., van der Kwast, T. H., Grignon, D., Egevad, L., Kristiansen, G., Pritchard, C. C., Rubin, M. A., & Bubendorf, L. (2020). Report From the International Society of Urological Pathology (ISUP) Consultation Conference on Molecular Pathology of Urogenital Cancers. I. Molecular Biomarkers in Prostate Cancer. *American Journal of Surgical Pathology*, *44*(7), e15–e29. https://doi.org/10.1097/PAS.0000000000001450

Ma, T., Yang, S., Jing, H., Cong, L., Cao, Z., Liu, Z., & Huang, Z. (2018). Apparent diffusion coefficients in prostate cancer: correlation with molecular markers Ki-67, HIF-1α and VEGF. *NMR in Biomedicine*, *31*(3), e3884. https://doi.org/10.1002/nbm.3884

Magi-Galluzzi, C. (2018). Prostate cancer: diagnostic criteria and role of immunohistochemistry. *Modern Pathology*, *31*(S1), 12–21. https://doi.org/10.1038/modpathol.2017.139

May, M., Knoll, N., Siegsmund, M., Fahlenkamp, D., Vogler, H., Hoschke, B., & Gralla, O. (2007). Validity of the CAPRA Score to Predict Biochemical Recurrence-Free Survival After Radical Prostatectomy. Results From a European Multicenter Survey of 1,296 Patients. *Journal of Urology*, *178*(5), 1957–1962. https://doi.org/10.1016/j.juro.2007.07.043

McGrath, S., Christidis, D., Perera, M., Hong, S. K., Manning, T., Vela, I., & Lawrentschuk, N. (2016). Prostate cancer biomarkers: Are we hitting the mark? *Prostate International*, *4*(4), 130–135. https://doi.org/10.1016/j.prnil.2016.07.002

McNally, C. J., Watt, J., Kurth, M. J., Lamont, J. v., Moore, T., Fitzgerald, P., Pandha, H., McKenna, D. J., & Ruddock, M. W. (2022). A Novel Combination of Serum Markers in a Multivariate Model to Help Triage Patients Into "Low-" and "High-Risk" Categories for Prostate Cancer. *Frontiers in Oncology*, *12*, 2100. https://doi.org/10.3389/fonc.2022.837127

Mistry, K., & Cable, G. (2003). Meta-Analysis of Prostate-Specific Antigen and Digital Rectal Examination as Screening Tests for Prostate Carcinoma. *The Journal of the American Board of Family Medicine*, *16*(2), 95–101. https://doi.org/10.3122/jabfm.16.2.95

Mitani, T., Harada, N., Nakano, Y., Inui, H., & Yamaji, R. (2012). Coordinated Action of Hypoxia-inducible Factor-1α and β-Catenin in Androgen Receptor Signaling. *Journal of Biological Chemistry*, *287*(40), 33594–33606. https://doi.org/10.1074/jbc.M112.388298

Nielsen, T. O., Leung, S. C. Y., Rimm, D. L., Dodson, A., Acs, B., Badve, S., Denkert, C., Ellis, M. J., Fineberg, S., Flowers, M., Kreipe, H. H., Laenkholm, A.-V., Pan, H., Penault-Llorca, F. M., Polley, M.-Y., Salgado, R., Smith, I. E., Sugie, T., Bartlett, J. M. S., … Hayes, D. F. (2021). Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. *JNCI: Journal of the National Cancer Institute*, *113*(7), 808–819. https://doi.org/10.1093/jnci/djaa201

Parker, C., Castro, E., Fizazi, K., Heidenreich, A., Ost, P., Procopio, G., Tombal, B., & Gillessen, S. (2020). Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *31*(9), 1119–1134. https://doi.org/10.1016/j.annonc.2020.06.011

Perner, S., Demichelis, F., Beroukhim, R., Schmidt, F. H., Mosquera, J.-M., Setlur, S., Tchinda, J., Tomlins, S. A., Hofer, M. D., Pienta, K. G., Kuefer, R., Vessella, R., Sun, X.-W., Meyerson, M., Lee, C., Sellers, W. R., Chinnaiyan, A. M., & Rubin, M. A. (2006). *TMPRSS2:ERG* Fusion-Associated Deletions Provide Insight into the Heterogeneity of Prostate Cancer. *Cancer Research*, *66*(17), 8337–8341. https://doi.org/10.1158/0008-5472.CAN-06-1482

Ranasinghe, W. K. B., Baldwin, G. S., Shulkes, A., Bolton, D., & Patel, O. (2014). Normoxic regulation of HIF-1α in prostate cancer. *Nature Reviews Urology*, *11*(7), 419–419. https://doi.org/10.1038/nrurol.2013.110-c2

Rawla, P. (2019). Epidemiology of Prostate Cancer. *World Journal of Oncology*, *10*(2), 63–89. https://doi.org/10.14740/wjon1191

Rebello, R. J., Oing, C., Knudsen, K. E., Loeb, S., Johnson, D. C., Reiter, R. E., Gillessen, S., van der Kwast, T., & Bristow, R. G. (2021). Prostate cancer. *Nature Reviews Disease Primers*, *7*(1), 9. https://doi.org/10.1038/s41572-020-00243-0

Remnant, L., Kochanova, N. Y., Reid, C., Cisneros-Soberanis, F., & Earnshaw, W. C. (2021). The intrinsically disorderly story of Ki-67. *Open Biology*, *11*(8), 210120. https://doi.org/10.1098/rsob.210120

Roobol, M. J. (2011). Contemporary role of prostate cancer gene 3 in the management of prostate cancer. *Current Opinion in Urology*, *21*(3), 225–229. https://doi.org/10.1097/MOU.0b013e328344939c

Sailer, V., Stephan, C., Wernert, N., Perner, S., Jung, K., Dietel, M., & Kristiansen, G. (2013). Comparison of p40 (ΔNp63) and p63 expression in prostate tissues - which one is the superior diagnostic marker for basal cells? *Histopathology*, *63*(1), 50–56. https://doi.org/10.1111/his.12116

Salami, S. S., Schmidt, F., Laxman, B., Regan, M. M., Rickman, D. S., Scherr, D., Bueti, G., Siddiqui, J., Tomlins, S. A., Wei, J. T., Chinnaiyan, A. M., Rubin, M. A., & Sanda, M. G. (2013). Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. *Urologic Oncology: Seminars and Original Investigations*, *31*(5), 566–571. https://doi.org/10.1016/j.urolonc.2011.04.001

Sanda, M. G., Cadeddu, J. A., Kirkby, E., Chen, R. C., Crispino, T., Fontanarosa, J., Freedland, S. J., Greene, K., Klotz, L. H., Makarov,

D. v., Nelson, J. B., Rodrigues, G., Sandler, H. M., Taplin, M. E., & Treadwell, J. R. (2018). Clinically Localized Prostate Cancer: AUA/ASTRO/SUO Guideline. Part I: Risk Stratification, Shared Decision Making, and Care Options. *Journal of Urology*, *199*(3), 683–690. https://doi.org/10.1016/j.juro.2017.11.095

Scholzen, T., & Gerdes, J. (2000). The Ki-67 protein: From the known and the unknown. *Journal of Cellular Physiology*, *182*(3), 311–322. https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

Sharp, A., Coleman, I., Yuan, W., Sprenger, C., Dolling, D., Rodrigues, D. N., Russo, J. W., Figueiredo, I., Bertan, C., Seed, G., Riisnaes, R., Uo, T., Neeb, A., Welti, J., Morrissey, C., Carreira, S., Luo, J., Nelson, P. S., Balk, S. P., … Plymate, S. R. (2018). Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. *Journal of Clinical Investigation*, *129*(1), 192–208. https://doi.org/10.1172/JCI122819

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians*, *72*(1), 7–33. https://doi.org/10.3322/caac.21708

Sobhani, N., Neeli, P. K., D'Angelo, A., Pittacolo, M., Sirico, M., Galli, I. C., Roviello, G., & Nesi, G. (2021). AR-V7 in Metastatic Prostate Cancer: A Strategy beyond Redemption. *International Journal of Molecular Sciences*, *22*(11), 5515. https://doi.org/10.3390/ijms22115515

Stephan, C., Jung, K., Semjonow, A., Schulze-Forster, K., Cammann, H., Hu, X., Meyer, H.-A., Bögemann, M., Miller, K., & Friedersdorff, F. (2013). Comparative Assessment of Urinary Prostate Cancer Antigen 3 and TMPRSS2:ERG Gene Fusion with the Serum [−2]Proprostate-Specific Antigen–Based Prostate Health Index for Detection of Prostate Cancer. *Clinical Chemistry*, *59*(1), 280–288. https://doi.org/10.1373/clinchem.2012.195560

Stephan, C., Ralla, B., & Jung, K. (2014). Prostate-specific antigen and other serum and urine markers in prostate cancer. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1846*(1), 99–112. https://doi.org/10.1016/j.bbcan.2014.04.001

Sun, X., & Kaufman, P. D. (2018). Ki-67: more than a proliferation marker. *Chromosoma*, *127*(2), 175–186. https://doi.org/10.1007/s00412-018-0659-8

Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G. A., Zelenetz, A. D., & Jaffe, E. S. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*, *127*(20), 2375–2390. https://doi.org/10.1182/blood-2016-01-643569

Szymańska, K., & Hainaut, P. (2018). Prostate Cancer: Diagnosis and Treatment. In *Reference Module in Biomedical Sciences* (pp. 292–298). Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.65828-2

Takagi, M., Ono, T., Natsume, T., Sakamoto, C., Nakao, M., Saitoh, N., Kanemaki, M. T., Hirano, T., & Imamoto, N. (2018). Ki-67 and condensins support the integrity of mitotic chromosomes through

distinct mechanisms. *Journal of Cell Science*, *131*(6). https://doi.org/10.1242/jcs.212092

Tomić, T. T., Gustavsson, H., Wang, W., Jennbacken, K., Welén, K., & Damber, J.-E. (2012). Castration resistant prostate cancer is associated with increased blood vessel stabilization and elevated levels of VEGF and Ang-2. *The Prostate*, *72*(7), 705–712. https://doi.org/10.1002/pros.21472

Tong, G., Zhang, G., Liu, J., Zheng, Z., Chen, Y., Niu, P., & Xu, X. (2020). Cutoff of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC-8 stratification. *Oncology Reports*, *43*(4), 1187. https://doi.org/10.3892/or.2020.7511

Truong, M., Yang, B., & Jarrard, D. F. (2013). Toward the Detection of Prostate Cancer in Urine: A Critical Analysis. *Journal of Urology*, *189*(2), 422–429. https://doi.org/10.1016/j.juro.2012.04.143

Uxa, S., Castillo-Binder, P., Kohler, R., Stangner, K., Müller, G. A., & Engeland, K. (2021). Ki-67 gene expression. *Cell Death & Differentiation*, *28*(12), 3357–3370. https://doi.org/10.1038/s41418-021-00823-x

Vickers, A. J., Savage, C., O'Brien, M. F., & Lilja, H. (2009). Systematic Review of Pretreatment Prostate-Specific Antigen Velocity and Doubling Time As Predictors for Prostate Cancer. *Journal of Clinical Oncology*, *27*(3), 398–403. https://doi.org/10.1200/JCO.2008.18.1685

Wang, K., Peng, H.-L., & Li, L.-K. (2012). Prognostic Value of Vascular Endothelial Growth Factor Expression in Patients with Prostate Cancer: a Systematic Review with Meta-analysis. *Asian Pacific Journal of Cancer Prevention*, *13*(11), 5665–5669. https://doi.org/10.7314/APJCP.2012.13.11.5665

Wang, L., Lu, B., He, M., Wang, Y., Wang, Z., & Du, L. (2022). Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. *Frontiers in Public Health*, *10*, 176. https://doi.org/10.3389/fpubh.2022.811044

Wei, D., Chen, W., Meng, R., Zhao, N., Zhang, X., Liao, D., & Chen, G. (2018). Augmented expression of Ki-67 is correlated with clinicopathological characteristics and prognosis for lung cancer patients: an up-dated systematic review and meta-analysis with 108 studies and 14,732 patients. *Respiratory Research*, *19*(1), 150. https://doi.org/10.1186/s12931-018-0843-7

Wollersheim, B. M., van Eenbergen, M., van Asselt, K. M., Fossion, L. M. C. L., Koldewijn, E. L., Oddens, J. R., Oomens, E. H., Wijsman, B. P., van de Poll-Franse, L. v., & Ezendam, N. P. M. (2021). Type of treatment, symptoms and patient satisfaction play an important role in primary care contact during prostate cancer follow-up: results from the population-based PROFILES registry. *BMC Family Practice*, *22*(1), 218. https://doi.org/10.1186/s12875-021-01567-w

Yang, C., Zhang, J., Ding, M., Xu, K., Li, L., Mao, L., & Zheng, J. (2018). Ki67 targeted strategies for cancer therapy. *Clinical and Translational Oncology*, *20*(5), 570–575. https://doi.org/10.1007/s12094-017-1774-3

Zaiem, F., Jerbi, R., Albanyan, O., Puccio, J., Kafri, Z., Yang, J., & Gabali, A. M. (2020). High Ki67 proliferation index but not cell-of-origin subtypes is associated with shorter overall survival in diffuse large B-cell lymphoma. *Avicenna Journal of Medicine*, *10*(04), 241–248. https://doi.org/10.4103/ajm.ajm_81_20

Zavala, V. A., Bracci, P. M., Carethers, J. M., Carvajal-Carmona, L., Coggins, N. B., Cruz-Correa, M. R., Davis, M., de Smith, A. J., Dutil, J., Figueiredo, J. C., Fox, R., Graves, K. D., Gomez, S. L., Llera, A., Neuhausen, S. L., Newman, L., Nguyen, T., Palmer, J. R., Palmer, N. R., … Fejerman, L. (2021). Cancer health disparities in racial/ethnic minorities in the United States. *British Journal of Cancer*, *124*(2), 315–332. https://doi.org/10.1038/s41416-020-01038-6

Kinra, P., & Malik, A. (2020). Ki 67: Are we counting it right? *Indian Journal of Pathology and Microbiology*, *63*(1), 98. https://doi.org/10.4103/IJPM.IJPM_770_19

Habibzadeh, F., Habibzadeh, P., & Yadollahie, M. (2016). On determining the most appropriate test cut-off value: the case of tests with continuous results. *Biochemia Medica*, *26*(3), 297–307. https://doi.org/10.11613/BM.2016.034

Berlin, A., Castro-Mesta, J. F., Rodriguez-Romo, L., Hernandez-Barajas, D., González-Guerrero, J. F., Rodríguez-Fernández, I. A., González-Conchas, G., Verdines-Perez, A., & Vera-Badillo, F. E. (2017). Prognostic role of Ki-67 score in localized prostate cancer: A systematic review and meta-analysis. *Urologic Oncology: Seminars and Original Investigations*, *35*(8), 499–506. https://doi.org/10.1016/j.urolonc.2017.05.004

Brawley, O. W. (2012). Trends in prostate cancer in the United States. *Journal of the National Cancer Institute - Monographs*, *45*, 152–156. https://doi.org/10.1093/jncimonographs/lgs035

Cooperberg, M., Pasta, D., Elkin, E., Litwin, M., Latini, D. M., Duchane, J., & Carroll, P. R. (2005). The UCSF Cancer of the Prostate Risk Assessment (CAPRA) Score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. *J Urol*, *173*(6), 1938–1942. https://doi.org/10.1097/01.ju.0000158155.33890.e7.The

Cooperberg, M. R., Moul, J. W., & Carroll, P. R. (2005). The changing face of prostate cancer. *Journal of Clinical Oncology*, *23*(32), 8146–8151. https://doi.org/10.1200/JCO.2005.02.9751

D'Amico, A. V., Whittington, R., Bruce Malkowicz, S., Schultz, D., Blank, K., Broderick, G. A., Tomaszewski, J. E., Renshaw, A. A., Kaplan, I., Beard, C. J., & Wein, A. (1998). Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *Journal of the American Medical Association*, *280*(11), 969–974. https://doi.org/10.1001/jama.280.11.969

Dzulkifli, F. A., Mashor, M. Y., & Jaafar, H. (2018). An overview of recent counting methods for Ki67 IHC staining. *Journal of Biomedical and Clinical Sciences (JBCS)*, *3*(2), 10–17.

Epstein, J. I., Egevad, L., Amin, M. B., Delahunt, B., Srigley, J. R., & Humphrey, P. A. (2016). The 2014 international society of urological pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma definition of grading patterns and proposal for a new grading system. *American Journal of Surgical Pathology*, *40*(2), 244–252. https://doi.org/10.1097/PAS.0000000000000530

Fantony, J. J., Howard, L. E., Csizmadi, I., Armstrong, A. J., Lark, A. L., Galet, C., Aronson, W. J., & Freedland, S. J. (2018). Is Ki67 prognostic for aggressive prostate cancer? A multicenter real-world study. *Biomarkers in Medicine*, *12*(7), 727–736. https://doi.org/10.2217/bmm-2017-0322

Hernandez, D. J., Nielsen, M. E., Han, M., & Partin, A. W. (2007). Contemporary Evaluation of the D'Amico Risk Classification of Prostate Cancer. *Urology*, *70*(5), 931–935. https://doi.org/10.1016/j.urology.2007.08.055

Hoedemaeker, R. F., Vis, A. N., & Van Der Kwast, T. H. (2000). Staging prostate cancer. *Microscopy Research and Technique*, *51*(5), 423–429. https://doi.org/10.1002/1097-0029(20001201)51:5<423::AID-JEMT4>3.0.CO;2-4

Kammerer-Jacquet, S. F., Ahmad, A., Møller, H., Sandu, H., Scardino, P., Soosay, G., Beltran, L., Cuzick, J., & Berney, D. M. (2019a). Ki-67 is an independent predictor of prostate cancer death in routine needle biopsy samples: proving utility for routine assessments. *Modern Pathology*, *32*(9), 1303–1309. https://doi.org/10.1038/s41379-019-0268-y

Li, R., Heydon, K., Hammond, M. E., Grignon, D. J., Roach, M., Wolkov, H. B., Sandler, H. M., Shipley, W. U., & Pollack, A. (2004). Ki-67 staining index predicts distant metastasis and survival in locally advanced prostate cancer treated with radiotherapy: An analysis of patients in Radiation Therapy Oncology Group Protocol 86-10. *Clinical Cancer Research*, *10*(12 I), 4118–4124. https://doi.org/10.1158/1078-0432.CCR-1052-03

Lobo, J., Rodrigues, Â., Antunes, L., Graça, I., Ramalho-Carvalho, J., Vieira, F. Q., Martins, A. T., Oliveira, J., Jerónimo, C., & Henrique, R. (2018). High immunoexpression of Ki67, EZH2, and SMYD3 in diagnostic prostate biopsies independently predicts outcome in patients with prostate cancer. *Urologic Oncology: Seminars and Original Investigations*, *36*(4), 161.e7-161.e17. https://doi.org/10.1016/j.urolonc.2017.10.028

Lughezzani, G., Budäus, L., Isbarn, H., Sun, M., Perrotte, P., Haese, A., Chun, F. K., Schlomm, T., Steuber, T., Heinzer, H., Huland, H., Montorsi, F., Graefen, M., & Karakiewicz, P. I. (2010). Head-to-Head Comparison of the Three Most Commonly Used Preoperative Models for Prediction of Biochemical Recurrence After Radical Prostatectomy. *European Urology*, *57*(4), 562–568. https://doi.org/10.1016/j.eururo.2009.12.003

Maia, R., Dos Santos, G. A., Reis, S., Viana, N. I., Pimenta, R., Guimarães, V. R., Recuero, S., Romão, P., Leite, K. R. M., Srougi, M., & Passerotti, C. C. (2022). Can we use Ki67 expression to predict prostate cancer aggressiveness? *Revista Do Colegio Brasileiro de Cirurgioes*, *49*, 1–7. https://doi.org/10.1590/0100-6991e-20223200-en

Pollack, A., Desilvio, M., Khor, L. Y., Li, R., Al-Saleem, T. I., Hammond, M. E., Venkatesan, V., Lawton, C. A., Roach, M., Shipley, W. U., Hanks, G. E., & Sandler, H. M. (2004). Ki-67 staining is a strong predictor of distant metastasis and mortality for men with prostate cancer treated with radiotherapy plus androgen deprivation: Radiation Therapy Oncology Group trial 92-02. *Journal of Clinical Oncology*, *22*(11), 2133–2140. https://doi.org/10.1200/JCO.2004.09.150

Pollack, A., Dignam, J. J., Diaz, D. A., Wu, Q., Stoyanova, R., Bae, K., Dicker, A. P., Sandler, H., Hanks, G. E., & Feng, F. Y. (2014). A Tissue Biomarker–Based Model That Identifies Patients with a High Risk of Distant Metastasis and Differential Survival by Length of Androgen Deprivation Therapy in RTOG Protocol 92-02. *Clinical Cancer Research*, *20*(24), 6379–6388. https://doi.org/10.1158/1078-0432.CCR-14-0075

Rawla, P. (2019). Epidemiology of Prostate Cancer. *World Journal of Oncology*, *10*(2), 63–89. https://doi.org/10.14740/wjon1191

Richardsen, E., Andersen, S., Al-Saad, S., Rakaee, M., Nordby, Y., Pedersen, M. I., Ness, N., Grindstad, T., Movik, I., Dønnem, T., Bremnes, R., & Busund, L. T. (2017). Evaluation of the proliferation marker Ki-67 in a large prostatectomy cohort. *PLoS ONE*, *12*(11). https://doi.org/10.1371/journal.pone.0186852

Sanda, M. G., Cadeddu, J. A., Kirkby, E., Chen, R. C., Crispino, T., Fontanarosa, J., Freedland, S. J., Greene, K., Klotz, L. H., Makarov, D. V., Nelson, J. B., Rodrigues, G., Sandler, H. M., Taplin, M. E., & Treadwell, J. R. (2018). Clinically Localized Prostate Cancer: AUA/ASTRO/SUO Guideline. Part I: Risk Stratification, Shared Decision Making, and Care Options. *Journal of Urology*, *199*(3), 683–690. https://doi.org/10.1016/j.juro.2017.11.095

Thompson, I. M., Pauler, D. K., Goodman, P. J., Tangen, C. M., Lucia, M. S., Parnes, H. L., Minasian, L. M., Ford, L. G., Lippman, S. M., Crawford, E. D., Crowley, J. J., & Coltman, C. A. (2004). Prevalence of Prostate Cancer among Men with a Prostate-Specific Antigen Level ≤4.0 ng per Milliliter. *New England Journal of Medicine*, *350*(22), 2239–2246. https://doi.org/10.1056/NEJMoa031918

Tollefson, M. K., Karnes, R. J., Kwon, E. D., Lohse, C. M., Rangel, L. J., Mynderse, L. A., Cheville, J. C., & Sebo, T. J. (2014). Prostate cancer Ki-67 (MIB-1) expression, perineural invasion, and gleason score as biopsy-based predictors of prostate cancer mortality: The mayo model. *Mayo Clinic Proceedings*, *89*(3), 308–318. https://doi.org/10.1016/j.mayocp.2013.12.001

Tretiakova, M. S., Wei, W., Boyer, H. D., Newcomb, L. F., Hawley, S., Auman, H., Vakar-Lopez, F., McKenney, J. K., Fazli, L., Simko, J., Troyer, D. A., Hurtado-Coll, A., Thompson, I. M., Carroll, P. R.,

Ellis, W. J., Gleave, M. E., Nelson, P. S., Lin, D. W., True, L. D., … Brooks, J. D. (2016). Prognostic value of Ki67 in localized prostate carcinoma: A multi-institutional study of >1000 prostatectomies. *Prostate Cancer and Prostatic Diseases*, *19*(3), 264–270. https://doi.org/10.1038/pcan.2016.12

Verhoven, B., Yan, Y., Ritter, M., Khor, L. Y., Hammond, E., Jones, C., Amin, M., Bahary, J. P., Zeitzer, K., & Pollack, A. (2013). Ki-67 is an independent predictor of metastasis and cause-specific mortality for prostate cancer patients treated on Radiation Therapy Oncology Group (RTOG) 94-08. *International Journal of Radiation Oncology Biology Physics*, *86*(2), 317–323. https://doi.org/10.1016/j.ijrobp.2013.01.016

Vlajnic, T., Brunner, P., Eppenberger-Castori, S., Rentsch, C. A., Zellweger, T., & Bubendorf, L. (2022). High Inter- and Intratumoral Variability of Ki67 Labeling Index in Newly Diagnosed Prostate Cancer with High Gleason Scores. *Pathobiology*, *89*(2), 74–80. https://doi.org/10.1159/000519007

Wei, L., Wang, J., Lampert, E., Schlanger, S., DePriest, A. D., Hu, Q., Gomez, E. C., Murakam, M., Glenn, S. T., Conroy, J., Morrison, C., Azabdaftari, G., Mohler, J. L., Liu, S., & Heemers, H. V. (2017). Intratumoral and Intertumoral Genomic Heterogeneity of Multifocal Localized Prostate Cancer Impacts Molecular Classifications and Genomic Prognosticators. *European Urology*, *71*(2), 183–192. https://doi.org/10.1016/j.eururo.2016.07.008

Wilkins, A. C., Gusterson, B., Szijgyarto, Z., Haviland, J., Griffin, C., Stuttle, C., Daley, F., Corbishley, C. M., Dearnaley, D. P., Hall, E., & Somaiah, N. (2018). Ki67 Is an Independent Predictor of Recurrence in the Largest Randomized Trial of 3 Radiation Fractionation Schedules in Localized Prostate Cancer. *International Journal of Radiation Oncology Biology Physics*, *101*(2), 309–315. https://doi.org/10.1016/j.ijrobp.2018.01.072

Zellweger, T., Günther, S., Zlobec, I., Savic, S., Sauter, G., Moch, H., Mattarelli, G., Eichenberger, T., Curschellas, E., Rüfenacht, H., Bachmann, A., Gasser, T. C., Mihatsch, M. J., & Bubendorf, L. (2009). Tumour growth fraction measured by immunohistochemical staining of Ki67 is an independent prognostic factor in preoperative prostate biopsies with small-volume or low-grade prostate cancer. *International Journal of Cancer*, *124*(9), 2116–2123. https://doi.org/10.1002/ijc.24174

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ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :31.03.2022

Toplantı No :2022/101

Proje No :1539

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Yrd. Doç. Dr. Fikret Dirilenoğlu'nun sorumlu araştırmacısı olduğu, YDU/2022/101-1539 proje numaralı ve **"Assessment of Immunohistochemical Ki-67 Expression in Grading Acinar Adenocarcinoma of the Prostate"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı

Yakın Doğu Üniversitesi

Bilimsel Araştırmalar Etik Kurulu Başkanı

Kurul Üyesi	**Toplantıya Katılım**	**Karar**
	Katıldı(✓)/ Katılmadı(X)	*Onay(✓)/ Ret(X)*
Prof. Dr. Tamer Yılmaz	*X*	*X*
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Nurhan Bayraktar	✓	✓
Prof. Dr. Mehmet Özmenoğlu	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
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Ishizaki, F., M. A. Hoque, T. Nishiyama, T. Kawasaki, T. Kasahara, N. Hara, I. Takizawa, T. Saito, Y. Kitamura, K. Akazawa, and K. Takahashi. "External Validation of the UCSF-CAPRA (University of California, San Francisco, Cancer of the Prostate Risk Assessment) in Japanese Patients Receiving Radical Prostatectomy", Japanese Journal of Clinical Oncology, 2011.
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Tamara L. Lotan, Scott A. Tomlins, Tarek A. Bismar, Theodorus H. Van der Kwast et al. "Report From the International Society of Urological Pathology (ISUP) Consultation Conference on Molecular Pathology of Urogenital Cancers. I. Molecular Biomarkers in Prostate Cancer", American Journal of Surgical Pathology, 2020
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https://www.ncbi.nlm.nih.gov/pubmed/27136741
<1% match ()

Amit Gupta, Sweety Gupta, Deepak Rajput, Prashant Durgapal et al. "Expression and clinicopathological correlation of Ki-67 in gallbladder carcinoma", Journal of Carcinogenesis
<1% match (Internet from 15-Oct-2022)

https://bura.brunel.ac.uk/bitstream/2438/16862/1/Fulltext.pdf
<1% match (Internet from 24-Sep-2022)

https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0186852
<1% match (Internet from 06-Feb-2020)

https://journals.plos.org/plosone/article/file?id=10.1371%2Fjournal.pone.0224671&type=printable
<1% match (Internet from 11-Jul-2022)

https://www.researchgate.net/publication/358645495_Prostate_Cancer_Incidence_and_Mortality_Global_Status_and_Temporal_Trends_in_89_Countries_From_2000_to_2019
<1% match (Internet from 07-Aug-2020)

https://kurume.repo.nii.ac.jp/index.php?action=pages_view_main&active_action=repository_action_common_download&attribute_id=17&block_id=21&file_no=2&item_id=951&item_no=1&page_id=13
<1% match (An Sen Tan, Joe Poe Sheng Yeong, Chi Peng Timothy Lai, Chong Hui Clara Ong et al. "The role of Ki-67 in Asian triple negative breast cancers: a novel combinatory panel approach", Virchows Archiv, 2019)

An Sen Tan, Joe Poe Sheng Yeong, Chi Peng Timothy Lai, Chong Hui Clara Ong et al. "The role of Ki-67 in Asian triple negative breast cancers: a novel combinatory panel approach", Virchows Archiv, 2019
<1% match (Internet from 29-Jul-2021)

https://archive.org/stream/vcb6567/Encyclopedias%20%28dratef.net%20%29%20%20Encyclopedia%20of%20Cancer%20%203%20Volume%20Set%203rd%20Edition%202019%20%20%28medical.shoppingdealer.com%20%29%20%20_djvu.txt
<1% match (Internet from 04-Jul-2019)

https://recipp.ipp.pt/handle/10400.22/14101
<1% match (Prostate Cancer A Comprehensive Perspective, 2013.)

Prostate Cancer A Comprehensive Perspective, 2013.
<1% match (Xuechao Liu, Haibo Qiu, Peng Zhang, Xingyu Feng, Tao Chen, Yong Li, Kaixiong Tao, Guoxin Li, Xiaowei Sun, Zhiwei Zhou. "Ki-67 labeling index may be a promising indicator to identify “very high risk” gastrointestinal stromal tumor (GIST): a multicenter retrospective study of 1022 patients", Human Pathology, 2017)

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<1% match (Teng Ma, Shaolin Yang, Haiyan Jing, Lin Cong, Zhixin Cao, Zhiling Liu, Zhaoqin Huang. "Apparent diffusion coefficients in prostate cancer: correlation with molecular markers Ki-67, HIF-1α and VEGF", NMR in Biomedicine, 2018)

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<1% match ()

McNally, Christopher, Watt, Joanne et al. "A Novel Combination of Serum Markers in a Multivariate Model to Help Triage Patients Into "Low-" and "High-Risk" Categories for Prostate Cancer", 'Frontiers Media SA', 2022
<1% match ()

Yoh, Kathryn Elizabeth. "Ras, p63 and breast cancer", 'Columbia University Libraries/Information Services', 2016
<1% match (Internet from 12-Aug-2022)

https://mdpi-res.com/d_attachment/cells/cells-11-02388/article_deploy/cells-11-02388.epub
<1% match (Internet from 01-Nov-2022)

https://mdpi-res.com/d_attachment/curroncol/curroncol-29-00640/article_deploy/curroncol-29-00640.epub
<1% match (Internet from 06-Sep-2017)

https://papyrus.bib.umontreal.ca/xmlui/bitstream/handle/1866/10867/Labouba_Ingrid_2013_these.pdf?isAllowed=y&sequence=2
<1% match (Internet from 12-Jan-2021)

http://theses.gla.ac.uk
<1% match (Internet from 23-Oct-2020)

https://www.agilent.com/en/product/dako-omnis-solution-for-ihc-ish/primary-antibodies-for-dako-omnis
<1% match (Internet from 30-May-2022)

https://www.mdpi.com/2076-2615/12/10/1263/htm
<1% match (Internet from 15-Dec-2015)

http://crs.or.jp
<1% match (Internet from 13-Oct-2022)

http://journal.waocp.org
<1% match (Akira Miyauchi, Takumi Kudo, Mitsuyoshi Hirokawa, Yasuhiro Ito et al. "Ki-67 Labeling Index Is a Predictor of Postoperative Persistent Disease and Cancer Growth and a Prognostic Indicator in Papillary Thyroid Carcinoma", European Thyroid Journal, 2013)

Akira Miyauchi, Takumi Kudo, Mitsuyoshi Hirokawa, Yasuhiro Ito et al. "Ki-67 Labeling Index Is a Predictor of Postoperative Persistent Disease and Cancer Growth and a Prognostic Indicator in Papillary Thyroid Carcinoma", European Thyroid Journal, 2013
<1% match ("Prostate Cancer Screening", Springer Science and Business Media LLC, 2009)

"Prostate Cancer Screening", Springer Science and Business Media LLC, 2009
<1% match (Jakobsen, Jan Nyrop, and Jens Benn Sørensen. "Clinical impact of ki-67 labeling index in non-small cell lung cancer", Lung Cancer, 2013.)

Jakobsen, Jan Nyrop, and Jens Benn Sørensen. "Clinical impact of ki-67 labeling index in non-small cell lung cancer", Lung Cancer, 2013.
<1% match (Internet from 06-Jan-2022)

https://bmcmedinformdecismak.biomedcentral.com/articles/10.1186/s12911-021-01732-2
<1% match (Internet from 28-Jan-2022)

https://core.ac.uk/download/194211938.pdf
<1% match (Internet from 26-Sep-2022)

https://open.uct.ac.za/bitstream/handle/11427/36672/thesis_hsf_2020_Vava_A.pdf;jsessionid=1BCCFB6A64EF4FBE26315FA71A9F4DCB?sequence=4
<1% match (Internet from 10-Dec-2021)

https://www.thefreelibrary.com/Contemporary+role+of+biomarkers+in+the+evaluation%2c+stratification%2c...-a0503465386
<1% match ()

Tharmalingam, Hannah. "Optimization of the radiotherapy management of patients with high-risk prostate cancer", 2020
<1% match (Internet from 09-Sep-2017)

http://www.zora.uzh.ch
<1% match ()

Archer, Leanne K. "The role of the ETS factor ELF3 in the normal and malignant prostate", University of York, 2018
<1% match (Pelosi, Giuseppe, Guido Rindi, William D. Travis, and Mauro Papotti. "Ki-67 Antigen in Lung Neuroendocrine Tumors : Unraveling a Role in Clinical Practice", Journal of Thoracic Oncology, 2014.)

Pelosi, Giuseppe, Guido Rindi, William D. Travis, and Mauro Papotti. "Ki-67 Antigen in Lung Neuroendocrine Tumors : Unraveling a Role in Clinical Practice", Journal of Thoracic Oncology, 2014.
<1% match (Internet from 19-Feb-2022)

https://ir.ymlib.yonsei.ac.kr/bitstream/22282913/184030/1/T202101909.pdf
<1% match (van der Poel, H.G.. "Molecular markers in the diagnosis of prostate cancer", Critical Reviews in Oncology and Hematology, 200702)

van der Poel, H.G.. "Molecular markers in the diagnosis of prostate cancer", Critical Reviews in Oncology and Hematology, 200702
<1% match ("Genitourinary", Laboratory Investigation, 2008)

"Genitourinary", Laboratory Investigation, 2008
<1% match ()

Powell, Katelyn Ann. "Tmprss2-Erg Regulation Of Androgen Biosynthetic Enzyme Expression, Dht Synthesis, And Androgen Receptor Activation In Prostate Cancer", DigitalCommons@WayneState, 2014
<1% match (Internet from 25-Sep-2022)

https://ec.europa.eu/environment/pdf/waste/elv/ELV%20Directive%20Implementation%20Report%202014-2017_Final%2027.06.19.pdf
<1% match (Internet from 25-Oct-2011)

http://gibk21.bio.kyutech.ac.jp
<1% match (Internet from 03-Mar-2022)

http://koreascience.kr
<1% match (Internet from 06-Dec-2021)

https://minerva-access.unimelb.edu.au/bitstream/handle/11343/261004/PMC5153438.pdf?isAllowed=y&sequence=1
<1% match (Internet from 26-Sep-2022)

https://oamjms.eu/index.php/mjms/article/download/oamjms.2018.309/2522/8332
<1% match (Internet from 15-Oct-2020)

https://www.hindawi.com/journals/bmri/2014/341324/
<1% match (Internet from 09-Aug-2022)

https://www.researchsquare.com/article/rs-1047551/v1
<1% match (Maryam Esfahani, Negar Ataei, Mojtaba Panjehpour. "Biomarkers for Evaluation of Prostate Cancer Prognosis", Asian Pacific Journal of Cancer Prevention, 2015)

Maryam Esfahani, Negar Ataei, Mojtaba Panjehpour. "Biomarkers for Evaluation of Prostate Cancer Prognosis", Asian Pacific Journal of Cancer Prevention, 2015
<1% match (Syed Harris Hussain, Cesar S. Huertas, Arnan Mitchell, Anne-Laure Deman, Emmanuelle Laurenceau. "Biosensors for circulating tumor cells (CTCs)-biomarker detection in lung and prostate cancer: Trends and prospects", Biosensors and Bioelectronics, 2021)

Syed Harris Hussain, Cesar S. Huertas, Arnan Mitchell, Anne-Laure Deman, Emmanuelle Laurenceau. "Biosensors for circulating tumor cells (CTCs)-biomarker detection in lung and prostate cancer: Trends and prospects", Biosensors and Bioelectronics, 2021
<1% match (Internet from 30-Apr-2020)

http://eprints.nottingham.ac.uk
<1% match ()

Kammerer-Jacquet, S-F, Ahmad, A, Møller, H, Sandu, H, Scardino, P, Soosay, G, Beltran, L, Cuzick, J, Berney, DM. "Ki-67 is an independent predictor of prostate cancer death in routine needle biopsy samples: proving utility for routine assessments.", Springer Nature
<1% match (Internet from 12-Dec-2021)

https://scholarworks.iu.edu/dspace/bitstream/handle/2022/25445/Final%20Dissertation.pdf?isAllowed=y&sequence=1
<1% match (Internet from 29-May-2018)

http://www.oncotarget.com
<1% match (Internet from 05-Jan-2022)

https://www.pubfacts.com/author/Eker%20O/1
<1% match ("General Methods and Overviews, Lung Carcinoma and Prostate Carcinoma", Springer Science and Business Media LLC, 2008)

"General Methods and Overviews, Lung Carcinoma and Prostate Carcinoma", Springer Science and Business Media LLC, 2008
<1% match (Internet from 04-Apr-2020)

https://ascopubs.org/doi/10.1200/JCO.2004.09.150
<1% match ()

Tokunaga Yoshiharu, Khalid Humayun, Hiura Tsuyoshi, Shibata Shobu. "Proliferation Potentials of Human Intracranial Neoplasms Assessed with Ki-67 (MIB-1) Labeling Index and Argyrophilic Nucleolar Organizer Regions", 1997
<1% match (Internet from 06-Oct-2022)

http://journalijdr.com
<1% match (Internet from 11-Dec-2019)

https://re.public.polimi.it/retrieve/handle/11311/1022158/202255/Grading%20lung%20neuroendocrine%20tumors_Controversies%20in%20search%20of%20a%20solution.pdf
<1% match (Internet from 11-Aug-2022)

https://repository.library.georgetown.edu/bitstream/handle/10822/1064615/Tyler_georgetown_0076M_15271.pdf?isAllowed=y&sequence=1
<1% match ("Precision Molecular Pathology of Prostate Cancer", Springer Science and Business Media LLC, 2018)

"Precision Molecular Pathology of Prostate Cancer", Springer Science and Business Media LLC, 2018
<1% match (Donna P. Ankerst. "Chapter 23 Pitfalls in Prostate Cancer Biomarker Evaluation Studies", Springer Science and Business Media LLC, 2009)

Donna P. Ankerst. "Chapter 23 Pitfalls in Prostate Cancer Biomarker Evaluation Studies", Springer Science and Business Media LLC, 2009
<1% match (Glen Kristiansen. "Diagnostic and prognostic molecular biomarkers for prostate cancer : Molecular biomarkers for prostate cancer", Histopathology, 01/2012)

Glen Kristiansen. "Diagnostic and prognostic molecular biomarkers for prostate cancer : Molecular biomarkers for prostate cancer", Histopathology, 01/2012
<1% match (M Losa. "Correlation between clinical characteristics and proliferative activity in patients with craniopharyngioma", Journal of Neurology, Neurosurgery & Psychiatry, 2004)

M Losa. "Correlation between clinical characteristics and proliferative activity in patients with craniopharyngioma", Journal of Neurology, Neurosurgery & Psychiatry, 2004
<1% match (Renjith P. Johnson, Chandrahas Koumar Ratnacaram, Lalit Kumar, Jobin Jose. "Combinatorial approaches of nanotherapeutics for inflammatory pathway targeted therapy of prostate cancer", Drug Resistance Updates, 2022)

Renjith P. Johnson, Chandrahas Koumar Ratnacaram, Lalit Kumar, Jobin Jose. "Combinatorial approaches of nanotherapeutics for inflammatory pathway targeted therapy of prostate cancer", Drug Resistance Updates, 2022
<1% match (S Jhavar, J Bartlett, G Kovacs, C Corbishley et al. "Biopsy tissue microarray study of Ki-67 expression in untreated, localized prostate cancer managed by active surveillance", Prostate Cancer and Prostatic Diseases, 2008)

S Jhavar, J Bartlett, G Kovacs, C Corbishley et al. "Biopsy tissue microarray study of Ki-67 expression in untreated, localized prostate cancer managed by active surveillance", Prostate Cancer and Prostatic Diseases, 2008
<1% match (V. Margulis. "Multi-Institutional Validation of the Predictive Value of Ki-67 Labeling Index in Patients With Urinary Bladder Cancer", JNCI Journal of the National Cancer Institute, 01/13/2009)

V. Margulis. "Multi-Institutional Validation of the Predictive Value of Ki-67 Labeling Index in Patients With Urinary Bladder Cancer", JNCI Journal of the National Cancer Institute, 01/13/2009
<1% match (Internet from 30-Sep-2022)

https://academic.oup.com/jncimono/article/2012/45/152/956507
<1% match (Internet from 20-Nov-2018)

http://docplayer.net
<1% match (Internet from 04-Oct-2022)

http://docs.neu.edu.tr
<1% match (Internet from 25-Jan-2022)

https://ecancer.org/es/journal/article/1063-the-capra-s-score-versus-subtypes-of-minimal-residual-disease-to-predict-biochemical-failure-after-radical-prostatectomy
<1% match (Internet from 31-Aug-2022)

https://edoc.ub.uni-muenchen.de/30380/1/Raj_Timsse.pdf
<1% match ()

Sun, Xiaoming. "Ki-67 Regulates Cell Cycle Progression and Heterochromatin Organization", eScholarship@UMMS, 2017
<1% match (Internet from 12-Sep-2022)

http://etd.uwc.ac.za
<1% match ()

Cosma, G, Acampora, G, Brown, D, Rees, RC, Khan, M, Pockley, AG. "Prediction of pathological stage in patients with prostate cancer: a neuro-fuzzy model", Public Library of Science
<1% match ()

Jefferies, Matthew. "Evaluating synergy between deregulation of the Wnt, PI3-Kinase and MAP-Kinase pathways in prostate tumourigenesis"
<1% match (Internet from 12-Nov-2022)

https://purehost.bath.ac.uk/ws/files/192788912/Dhafer_PhD_thesis_final_June_2019.pdf
<1% match ()

João António Ramalho Carvalho. "MicroRNAs deregulation in prostate cancer", 2017
<1% match (Internet from 24-Oct-2022)

http://repository.essex.ac.uk
<1% match (Internet from 20-Sep-2014)

http://www.doria.fi

<1% match (Internet from 21-Feb-2021)

https://www.karger.com/Article/FullText/477545

<1% match (Érica Romão Pereira, Amanda Letícia Francelino, Laís Capelasso Lucas Pinheiro, Carlos Alberto Miqueloto et al. "Tissue Immunostaining of Candidate Prognostic Proteins in Metastatic and Non-metastatic Prostate Cancer", Research Square Platform LLC, 2022)

Érica Romão Pereira, Amanda Letícia Francelino, Laís Capelasso Lucas Pinheiro, Carlos Alberto Miqueloto et al. "Tissue Immunostaining of Candidate Prognostic Proteins in Metastatic and Non-metastatic Prostate Cancer", Research Square Platform LLC, 2022

<1% match (Bret Verhoven, Yan Yan, Mark Ritter, Li-Yan Khor et al. "Ki-67 Is an Independent Predictor of Metastasis and Cause-Specific Mortality for Prostate Cancer Patients Treated on Radiation Therapy Oncology Group (RTOG) 94-08", International Journal of Radiation Oncology*Biology*Physics, 2013)

Bret Verhoven, Yan Yan, Mark Ritter, Li-Yan Khor et al. "Ki-67 Is an Independent Predictor of Metastasis and Cause-Specific Mortality for Prostate Cancer Patients Treated on Radiation Therapy Oncology Group (RTOG) 94-08", International Journal of Radiation Oncology*Biology*Physics, 2013

<1% match ("Prostate Cancer", Springer Science and Business Media LLC, 2018)

"Prostate Cancer", Springer Science and Business Media LLC, 2018

<1% match (David G. Bostwick, Liang Cheng. "Neoplasms of the Prostate", Elsevier BV, 2020)

David G. Bostwick, Liang Cheng. "Neoplasms of the Prostate", Elsevier BV, 2020

<1% match (Guillermo Lorenzo, Nadia di Muzio, Chiara Lucrezia Deantoni, Cesare Cozzarini et al. "Patient-specific forecasting of post-radiotherapy prostate-specific antigen kinetics enables early prediction of biochemical relapse", Cold Spring Harbor Laboratory, 2022)

Guillermo Lorenzo, Nadia di Muzio, Chiara Lucrezia Deantoni, Cesare Cozzarini et al. "Patient-specific forecasting of post-radiotherapy prostate-specific antigen kinetics enables early prediction of biochemical relapse", Cold Spring Harbor Laboratory, 2022

<1% match (Meurs, Pieter, Rose Galvin, Deirdre M. Fanning, and Tom Fahey. "Prognostic value of the CAPRA clinical prediction rule: a systematic review and meta-analysis : Prognostic value of the CAPRA clinical prediction rule", BJU International, 2013.)

Meurs, Pieter, Rose Galvin, Deirdre M. Fanning, and Tom Fahey. "Prognostic value of the CAPRA clinical prediction rule: a systematic review and meta-analysis : Prognostic value of the CAPRA clinical prediction rule", BJU International, 2013.

<1% match (Nuria Andrés-Sánchez, Daniel Fisher, Liliana Krasinska. "Physiological functions and roles in cancer of the proliferation marker Ki-67", Journal of Cell Science, 2022)

Nuria Andrés-Sánchez, Daniel Fisher, Liliana Krasinska. "Physiological functions and roles in cancer of the proliferation marker Ki-67", Journal of Cell Science, 2022

<1% match (Tobias Zellweger. "Tumour growth fraction measured by immunohistochemical staining of Ki67 is an independent prognostic factor in preoperative prostate biopsies with small-volume or low-grade prostate cancer", International Journal of Cancer, 05/01/2009)

Tobias Zellweger. "Tumour growth fraction measured by immunohistochemical staining of Ki67 is an independent prognostic factor in preoperative prostate biopsies with small-volume or low-grade prostate cancer", International Journal of Cancer, 05/01/2009
<1% match (Xiaoming Sun, Paul D. Kaufman. "Ki-67: more than a proliferation marker", Chromosoma, 2018)

Xiaoming Sun, Paul D. Kaufman. "Ki-67: more than a proliferation marker", Chromosoma, 2018
<1% match (Yongzhan Zhang, Lu Bai, Xiao-jun Huang, Ai-dong Lu et al. "Allogeneic Hematopoietic Stem Cell Transplantation, Especially Haploidentical, May Improve Long-term Survival for Children With High-risk T-cell Acute Lymphoblastic Leukemia in First Complete Remission", Research Square Platform LLC, 2021)

Yongzhan Zhang, Lu Bai, Xiao-jun Huang, Ai-dong Lu et al. "Allogeneic Hematopoietic Stem Cell Transplantation, Especially Haploidentical, May Improve Long-term Survival for Children With High-risk T-cell Acute Lymphoblastic Leukemia in First Complete Remission", Research Square Platform LLC, 2021

CV

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EMPLOYMENT HISTORY

08/2017 - 02/2021 Occupational Therapy Clinic

Patients' appointments coordination

Organize and monitor patients' files through dedicated software

Execution of the administrative and accounting affairs

08/2015 - 06/2017 **Maragheh University**

Work experience 2

Preparation of a variety of microbial culture media to isolate bacteria and packaging Preparation of gram staining kits,

Producing: vinegar, acid, alcohol and soap using an industrial and sterile methodology, Familiarization with laboratory animals, Performance of all laboratory safety tests, PCR, and gram staining of a variation of bacteria, Preparation of a variety of laboratory buffers, Identification of unknown bacteria, Separation of a variety of bacteria from varying environments, Preparation of gel electrophoresis and use of UV device, Microbial culture preparation, Anatomy of animals like doves, mice, frogs, fish and statical analysis.

08/2014 - 06/2015 **Maragheh University**

Work experience 1

Compilation of relevant and required letters and science magazine

Compilation, codification, and edition of relative subjects in publications and journals

Execution of cultural and educational affairs

Project

There are two projects on the expression analysis of 2

micro-RNA in prostate cancer, which were successfully completed, but it has not been published because of a small number of samples.

During the research
Preparation of samples of tissue and blood

Laboratory activities of the project include
Synthesis of cDNA, extraction of RNA and DNA from tissues and blood, working with devices such as autoclave, bain-marie, incubator, centrifugation, preparation of the samples to prove sample consolidation on carbon nanotubes and microplates and various materials using
AFM, EDX, SEM devices, time detection and optimum concentration with respect to the numbers given by citation 3, Analysis of elements of RNA and DNA molecules by spectrophotometer.

EDUCATIONAL QUALIFICATIONS

Apr 2021 – Nov 2022	Master of Medical Biology and Genetics (NEU university)
Sep 2012 - Aug 2016	Bachelor of Science in Microbiology
13th February 2016	HSE one-day course
25th February 2014	Laboratory Safety course
20th February 2014	ELISA one-day course
2nd - 4th Nov 2013	Participation in the Festival of Harkat
Sep 2010 - Jun 2011	Narjes Pre-University certificate in Experimental Science
Sept 2007 - Jun 2010	Asyieh High School in Experimental Science

*Master's certificate is taken from North Cyprus University. Yet others have been achieved in Iran

PERSONAL ATTRIBUTES	Independent character, energetic, solid work, creative, humble, kind, generous, punctual, well organized
SKILLS	Expertise in Microsoft Word, Excel, PowerPoint, Access
INTERESTS	Poetry, Science fiction, Painting, Cycling, Horse riding, Volleyball, Music