

Correlation of PTEN Losses by Immunohistochemistry and Fish in Prostate Adenocarcinoma

Batool Jameel Jubair¹, Sazan Abdulwahab Mirza Al-Atroshi²

¹Assistant lecturer, M.B.CH. B/MSc. (PATH), Collage of Medicine/ Al-Anbar University.

²Assistant professor, M.B.CH. B / F.I.C.M.S (PATH), Collage of Medicine/University of Baghdad.

Abstract

Background: Prostate cancer (PC) is the largest and most unusual site of robust neoplasm in Europe, accounting for 22.8% of all newly diagnosed cancers in men. An increase in the prevalence of pentachloro anisole has been observed in most countries. This may be explained by the massive use of prostate unique antigen (PSA) in the early detection of PCa in the early stages. **Objective:** Detection of PTEN loss by IHC and FISH technique and compare between the two methods and correlate with Gleason grading system. **Patients and method:** A retrospective sample study was performed. From January 2014 - Joe. 2019. This study included 120 cases of prostate cancer from Baghdad and Duhok, with different age groups. Fixed formalin paraffin biopsy for prostatectomy in cases obtained from the Archives of Pathology Laboratories in Baghdad” (Shahid Ghazi Hariri Training Hospital and Laboratories).

Results: PTEN (FISH) detected the gene in 29 case and confirmed by PTEN (IHC) expression results, underestimation occurred in five case detected by PTEN (IHC). Concerning PTEN (IHC), it detected the gene in 34 case. Percentage of cases showing negative staining for PTEN protein in high grade was higher than cases showing positive protein expression (good correlation with new grading group). IHC to detect PTEN protein levels is an interesting alternative to knowledge of the FISH PTEN gene loss and as a prognostic biomarker. There was significant association between PTEN gene expression and age. PTEN (IHC) showed negative results in 65.3% of case; while loss was shown in 58.2% of s by PTEN (FISH).

Conclusion: The important result in this study was a substantial agreement between the expression results of PTEN (FISH) and PTEN (IHC).

Keyword: PTEN (FISH), PTEN (IHC), Prostate adenocarcinoma, immunohistochemistry

Introduction

Prostate cancer (PCa) is the largest and most unusual site of solid neoplasm in Europe, accounting for 22.8% of all newly diagnosed cancers in men.⁽¹⁾ An increase in the prevalence of PCa has been observed in most countries. This may be explained by the massive use of prostate unique antigen (PSA) for detection of PCa in early stages.^(2,3)

In Iraq, prostate cancers represent the seventh most common malignancy in Iraq Registry for 2012.

⁽⁴⁾ Most prostate cancers are detected by virtual rectal exam (DRE), and transurethral ultrasound, however, ultrasound misses almost 30% of isoechoic carcinomas), or PSA improvement (both above 4ng / dL and growing over time). Nowadays, needle biopsy identifies several prostate cancers. Less often – its miles recognized by transurethral resection samples. Prostate cancers can be classified into:

(1) Adenocarcinomas (“secondary”) of peripheral ducts and acini ,

(2) large (“primary”) ductal carcinomas.

Roughly speaking, a lump can be hard to look, but it can usually be diagnosed as a poorly defined gray or

Corresponding author:

Dr. Batool Jameel Jubair

Email: pathology.department2016@gmail.com

yellow area, poorly delineated or corporation area.

Early detection tasks can detect minor tumors. ⁽⁵⁾

Microscopically: The four basic structures of cellular architecture patterns are the medium sized glands, little glands, diffuse single infiltration of cells and the cribriform form.

It is accompanied by a cytological incongruity in the nuclear enlargement, contour irregularity, hyperpigmentation and most importantly notable nucleoli (“macro-nucleoli”, Calibration > 1 µm in diameter). Nucleoli are typically numerous and more likely to be emarginated. Mitosis also important, however, it is rare in a well-differentiated tumor. ⁽⁵⁾

The types of prostate cancer that form glands are usually lined with a single cell layer, but rarely contain stratified epithelial tissue that mimics PIN ⁽⁶⁾ and have many benign mimics ⁽⁷⁾

Materials and Methods

During the period from January, 2014 to June 2019, a retrospective selective study was performed included 120 cases of prostate cancer with different age groups from Baghdad and Duhok.

Fixed formalin paraffin biopsy for prostatectomy obtained from the Archives of Pathology Laboratories in Baghdad” (Shahid Ghazi Hariri Teaching Hospital and Laboratories, Duhok Central Health laboratory and Vajeen specialist laboratory & certain private clinical laboratories.

Additionally, normal prostate in the form of paraffin blocks is a positive control (PTEN), and kidneys with RCC is a positive control (AMACR).

Patients’ clinical data, including age and provisional clinical diagnosis, were obtained from archival histological reports.

Each H&E stained slide was reviewed for pathological evaluation and diagnostic evaluation to confirm the diagnosis of prostate cancer. Cases are scored and graded in accordance with new grading.

Immunohistochemical technique and molecular work of the study were performed in Duhok (Vajeen

specialist laboratory).

Inclusion Criteria

Various types of prostate specimens were included in this study, including transurethral resection of the prostate, needle biopsy, and resection of the prostate.

Exclusion criteria:

Insufficient biopsies, poorly preserved prostate specimens, and biopsies with acute inflammation and necrosis were excluded.

Tissue Microarray Technology (TMA):

A new technique used in this study which is Tissue-Microarray

The procedure involves using a hollow needle to cut core of tissue just 2 mm in diameter from the areas of interest (previously detected prostate tumor areas in stained glass (H&E) slides) into paraffin embedded tissue.

Sections of the Microarray blocks are excised by a microtome, equine on a single microscope slide, then evaluated by staining with H and E, then another 4 µm section is made for the immunohisto-chemical stain for PTEN and one section for the FISH probe.

Immunohistochemical technique: Agilent Company ® .

A. The following equipment was used during the study For IHC:

- Microtome
- Water bath
- Glass slides.
- Microwave oven.
- Humidity chamber
- Optical microscope.
- FLEX IHC microscope slides (code K8020)
- Silanized chip (code S3003)
- Buffer solution - phosphate saline (PBS).

- xylene.
- Envision™ FLEX Hematoxylin (counter stain) Link (code K8008).
- Distilled water.
- Ethanol
- Aqueous mounting medium
- Permanent mounting medium
- Dako Autostainer / Autostainer Plus and Dako PT Link Tools.

B. PTEN Primary Antibody:

- monoclonal mouse against human clone PTEN 6H2.1, catalog number M3627; Made in Daku, USA.

Immunohistochemical method:

Immunohistochemical work in this study was performed with an automatic Link 48 autosstainer.

Micron sections were obtained from formalin-fixed and paraffin-embedded tissue blocks, a section utilizing Dako PT 3-in-1 specimen preparation procedure. Follow the pre-treatment procedures posted in the EnVision™ FLEX Target retrieval Solutions. Height of PH (10x) and PH 9 (code S2388) for PTEN.

Post staining the sections, they were dried, cleaned and impregnated with a permanent fixing medium.

Fluorescence *in Situ* (FISH) technique:

A. Equipment and materials required to study FISH in this research:

Zytolight FISH Tissue Incorporation Kit (serial number Z-2028-5 / -20.

Positive and negative control specimens

Positively charged slides

Water bath (37 ° C - 98 ° C)

Hybridization / hotplate

Hybridization or humidity chamber in the hybridization oven

Adjustable pipettes (10 ml, 25 ml)

Staining: cans or baths

Timers

Calibrated thermometer

Xylene

Distilled or Deionized water

Cover-slips (22 mm x 24 mm x 60 mm)

Rubber glue, such as B. fixogum cement-based rubber.

Fluorescence microscope (400-1000X)

Certified immersion oil for fluorescence microscopy

Appropriate filter sets

Reagent provided:

The Zytolight SPEC PTEN / CEN 10 dial probe consists of:

ZyGreen-labeled polynucleotide (excitation 503 nm / irradiance 528) (10 ng / mL, target sequence mapping in 10q23.2-q23.31 * (chr 10: 89,440,649-89,755,790) contains a region of PTEN gene

ZyOrang-labeled (547 nm excitation / 572 nm irradiance) polynucleotide (1.5 ng / mL) targeting the sequences shown in the 10p11.1-q11.1 of the alpha satellite central region of the D10Z1 chromosome 10.

Hybridized Buffer based on formamedia.

Counter –stain vial:300 µl per vial (30 tests). The counterstain is DAPI antifade (ES: 0.125 µg / ml DAPI (4,6-Diamidino-2-phenylindole)).

Cytocell comp. manufactured a pre-treatment kit and a removal kit.

B. Fluorescence in the in situ hybridization method:

Preparation of solutions used in FISH workings:

Sodium chloride sodium citrate (SSC solutions):

- **Sample preparation:**

- A section of tissue placed on a charged slide, then preserved in the oven at 55 ° C overnight to remove paraffin

- Then dehydrated in the next day

Pretreatment of tissue samples:

It consists of two stages:

1. Heat pre-treatment

2. Enzymes-Digestion

The samples then dried at room temperature in a 75%, 85% and 100% series of ethanol for 2 minutes at each concentration. Keep the slide for air drying and proceed to predenaturation, denaturation and hybridization.

FISH signaling analysis:

A fluorescent microscope should be available. Use the DAPI / FITC / Texas Red triple-band filter for optimal green, red, and DAPI fluorescent imaging.

Normal state: In interphases of normal cells or cells while not a deletion involving the PTEN gene region, two green and 2 orange signals appear.

Aberrant situation: In a cell with deletion affecting the PTEN gene region, a reduced number of green signals will be detected. Deletion affecting only parts

of the PTEN gene region might result in normal signal pattern with green signals of reduced size

statistical analysis:

All statistical procedures, data management and analyses were performed with the aid of the SPSS,IBM,US, version 25, The Kappa Cohen value was used to assess the degree of consistency and agreement of PTEN expression results FISH and PTEN(IHC). All analyses done under the assumption that p. value was two tailed and ≤ 0.05 to be considered significant

Results

A total of cases 98 cases with proved diagnosed adenocarcinomas were enrolled in this study.

Age Distribution

As it shown in figure 1, age distribution of the cases revealed that a mean age of the studied group was 70.15 ± 8.8 (range: 50-95) years. Higher proportion of cases aged 70 years or older accounted for (60.2%) of cases.

Figure 1: Age distribution of the studied group PTEN (IHC and FISH)

Results of PTEN test using immunohistochemistry and FISH are shown in table 1. PTEN (IHC) showed negative results in 65.3% of cases; while the loss was detected at 58.2% by PTEN (FISH).

Table 1: PTEN results by immunohistochemistry and FISH

a variable	No (n = 98)	percentage (%)
PTEN (IHC)		
negative	64	65.3
positive	34	34.7
PTEN (FISH)		
Loss	57	58.2
No losses	41	41.8

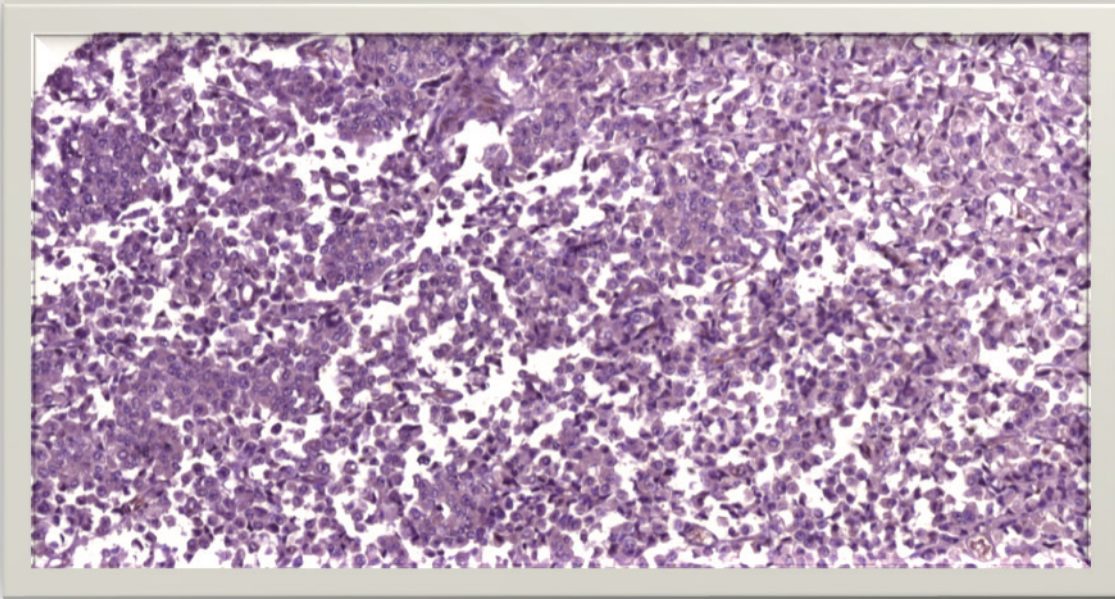


Figure 2 negative staining PTEN immunohistochemistry prostatic adenocarcinoma score 9 (4+5) Grade 5

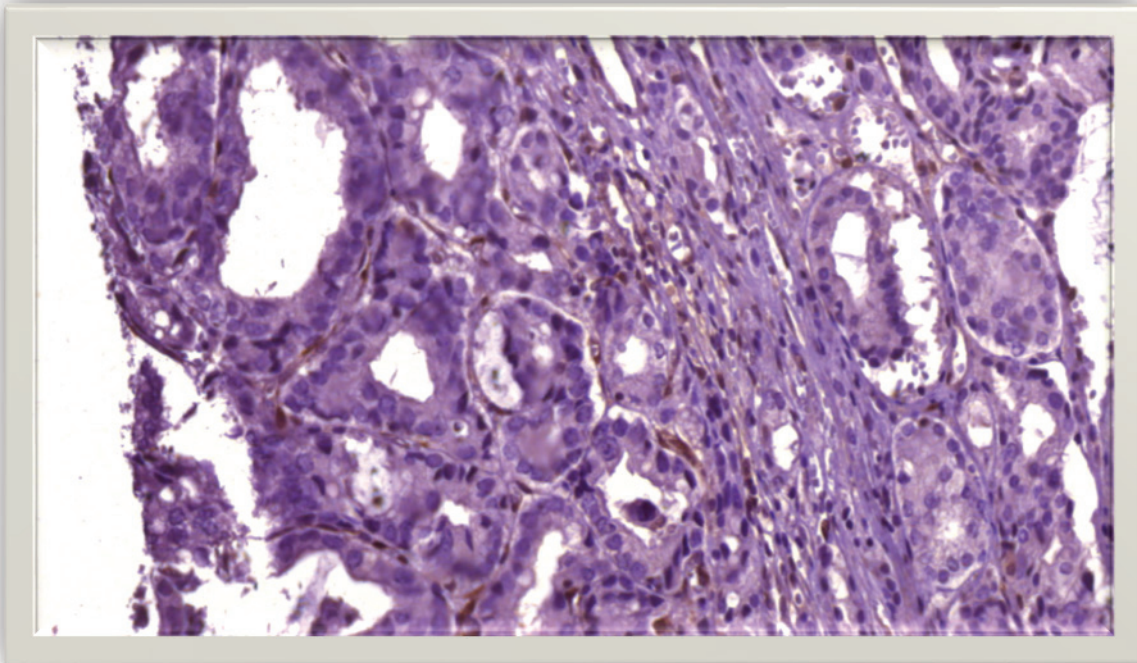


Figure 3 negative staining PTEN immunohistochemistry with internal positive control of PTEN benign prostatic hyperplasia

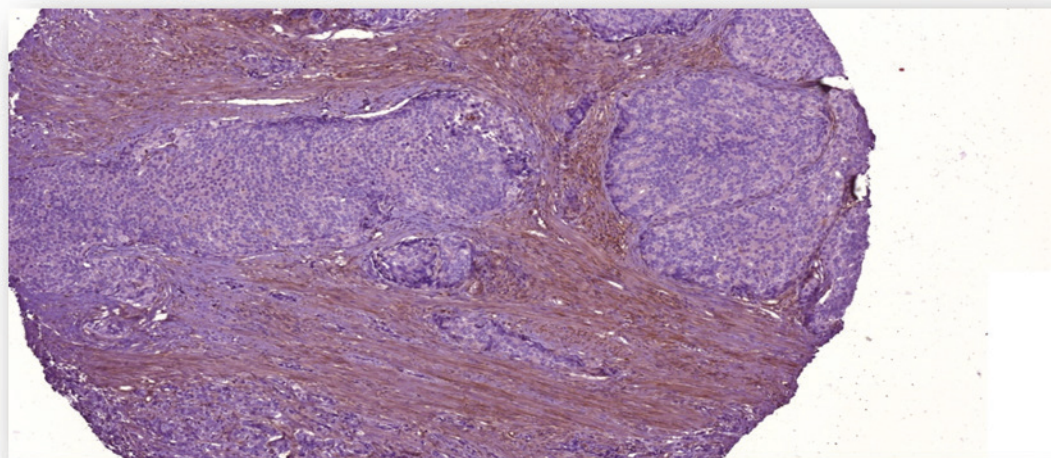


Fig 4: high grade adenocarcinoma of the prostate (Gleason grade 4+5=9) with urinary bladder metastasis. PTEN –VE .

PTEN (FISH) detected the gene in 29 case and confirmed by PTEN (IHC) expression results, underestimation occurred in five case detected by PTEN (IHC). Concerning PTEN (IHC), it detected the gene in 34 case. Percentage of cases showing negative staining for PTEN protein in high grade was higher than cases showing positive protein expression (good correlation with new grading group). IHC to detect PTEN protein levels is an interesting alternative to knowledge of the FISH PTEN gene loss and as a prognostic biomarker. There was significant association between PTEN gene expression and age.

Relationship between PTEN expression (IHC) and PTEN gene expression by (FISH).

PTEN (FISH) detected the gene in 29 cases and was confirmed by the results of PTEN (IHC) expression. Underestimation occurred in five cases (detected by PTEN (IHC). As for PTEN (IHC), the gene was detected in 34 cases.

During tissue sections, a part of the cell and the nucleus can be cut, resulting in a “truncation effect” in which loss of the PTEN signal from the nucleus can be erroneously estimated as deletion of the gene. Therefore, it is very important that false positive results, which were probably the result of truncation, were determined by comparison with normal nuclei in all FISH deletion tests. ⁽⁸⁾

Another explanation for the false negative result is a technical error in the FISH procedure due to air bubbles while the cap is slipping. This prevents the probe from reaching the desired area

Twelve cases showed presence of a gene by FISH procedure, which is not confirmed by immunochemotherapy, in fact there were two explanations for this finding, the first cause may be exposure to microRNA (**miR-4534 directly represses the tumor inhibitor PTEN gene**)The second is due to a point mutation in 10q23.3. ⁽⁹⁾,

An important outcome in this study was the significant substantial agreement between PTEN (FISH) and PTEN (IHC) expression results, and this agreement was statistically significant (kappa = 0.635, P = 0.001).

The PTEN (IHC) sensitivity was 70.7%, specificity 91.2%, and accuracy 82.7%.

In the same direction with this study, another study found that low PTEN expression was significantly associated with PTEN genomic removal which indicated that erasure is the main procedure erasures as the main method causing reduced PTEN protein expression .

In 28/39 cases (72%), a similarity was observed between deletion state of PTEN and lower expression of PTEN protein . Moreover, in eight cases out of eleven disagreement cases, grade I (declined) or grade

0 (absent) expression of PTEN protein were detected, these 8 cases were negative for PTEN deletion. While normal expression of PTEN was reported in the other 3 cases.⁽¹⁰⁾

The possibility that PTEN FISH might not recognize some cases of prostate cancer with PTEN inactivated greatly supports the need for replacement analysis to characterize PTEN loss.

IHC for detection of PTEN protein levels is a clear alternative for detecting PTEN FISH gene loss. Four previous studies assessed the usefulness of PTEN-IHC in prostate cancer as predictor of loss of PTEN.⁽¹¹⁻¹⁴⁾

some of which are currently in clinical trials for prostate cancer. PTEN loss could also be a biomarker of resistance to hormone therapy in advanced prostate cancer.⁽¹⁵⁾ The opportunity that PTEN FISH may fail to distinguish some cases of cancer of the prostate with PTEN deactivation powerfully maintains for the need for a

The PTEN protein expression by IHC is a simple analysis that is easier and cheaper to perform than FISH. As It is compared to FISH, this test is easier to be performed by needle biopsy-sampling and may indicates PTEN protein absence among additional cases. Therefore, when there PTEN protein is lack or absent on needle-biopsy specimens it could be a promising predictive biomarker that is useful in screening patients with low risk prostatic cancer who are likely to progress, where there will be a need to treatment and intervention.⁽¹⁵⁾

Moreover, loss of PTEN as a biopredictive marker may help select appropriate patients for treatment with Novel-PI 3-kinase pathway therapies, nonetheless, currently these therapies are under investigations by different clinical trials for treatment of prostate cancer. From other point of view, in advanced cases of prostate cancer, loss of PTEN can be used as an indicator-biomarker to predict resistance to hormonal therapy.⁽¹⁶⁾

Conclusion

The important result in this study was a substantial agreement between the expression results of PTEN (FISH) and PTEN (IHC).

No Conflicts of Interest

Source of Funding: self

Ethical Clearance: was taken from the scientific committee of the Iraqi Ministry of health

References

1. Rawla P. Epidemiology of prostate cancer. World journal of oncology. 2019 Apr;10(2):63.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. European journal of cancer. 2013 Apr 1;49(6):1374-403.
3. Bray F, Lortet-Tieulent J, Ferlay J, Forman D, Auvinen A. Prostate cancer incidence and mortality trends in 37 European countries: an overview. European journal of cancer. 2010 Nov 1;46(17):3040-52.
4. Al-Faluji AA, Ali SH, Al-Esawi AA. Incidence of cancer in Fallujah above 10 years age with over view of common cancers in 2011. Health. 2012 Sep 28;4(9):591-6.
5. Rosai J. Rosai and Ackerman's surgical pathology e-book. Elsevier Health Sciences; 2011 Jun 20.
6. Hameed O, Humphrey PA. Stratified epithelium in prostatic adenocarcinoma: a mimic of high-grade prostatic intraepithelial neoplasia. Modern pathology. 2006 Jul;19(7):899-906.
7. Zynger D, Parwani A. Prostate pathology. Demos Medical Publishing; 2014 Aug 14.
8. Nip H, Dar AA, Saini S, Colden M, Varahram S, Chowdhary H, Yamamura S, Mitsui Y, Tanaka Y, Kato T, Hashimoto Y. Oncogenic microRNA-4534 regulates PTEN pathway in prostate cancer. Oncotarget. 2016 Oct 18;7(42):68371.
9. Phin S, Moore M, Cotter PD. Genomic rearrangements of PTEN in prostate cancer. Frontiers in oncology. 2013 Sep 17;3:240.
10. Han B, Mehra R, Lonigro RJ, Wang L, Suleman K, Menon A, Palanisamy N, Tomlins SA, Chinnaiyan AM, Shah RB. Fluorescence in situ hybridization study shows association of PTEN deletion with ERG rearrangement during prostate cancer progression. Modern pathology. 2009 Aug;22(8):1083-93.
11. EPSTEIN JI, Potter SR. The pathological interpretation and significance of prostate

- needle biopsy findings: implications and current controversies. *The Journal of urology*. 2001 Aug 1;166(2):402-10.
12. Gaudin PB, Reuter VE. Benign mimics of prostatic adenocarcinoma on needle biopsy. *Anatomic pathology (Chicago, Ill.: annual)*. 1997 Jan 1;2:111-34.
 13. Epstein JI, Walsh PC, Carmichael M, Brendler CB. Pathologic and clinical findings to predict tumor extent of nonpalpable (stage t1 c) prostate cancer. *Jama*. 1994 Feb 2;271(5):368-74.
 14. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer research*. 1999 Sep 1;59(17):4291-6.
 15. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, Hicks JL, Park BH, Humphreys E, Partin AW, Han M. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clinical cancer research*. 2011 Oct 15;17(20):6563-73.
 16. Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, Lotan TL. Clinical implications of PTEN loss in prostate cancer. *Nature Reviews Urology*. 2018 Apr;15(4):222.