The Role of Immunohistochemical Analysis as a Tool for the Diagnosis, Prognostic Evaluation and Treatment of Prostate Cancer: A Systematic Review of the Literature

Arie Carneiro [1,2†], Álan Roger Gomes Barbosa [1†], Lucas Seiti Takemura [1], Paulo Priante Kayano [1], Natasha Kouvaleski Saviano Moran [2], Carolina Ko Chen [1], Marcelo Langer Wroclawski [1,2], Gustavo Caserta Lemos [1], Isabela Werneck da Cunha [3], Marcos Takeo Obara [4], Marcos Tobias-Machado [2], Adam G. Sowalsky [5] and Bianca Bianco [6*]

[1] Department of Urology, Hospital Israelita Albert Einstein, São Paulo, Brazil, [2] Department of Pathology, Hospital Israelita Albert Einstein, São Paulo, Brazil, [3] Department of Pathology, Rede D'OR São Luiz, São Paulo, Brazil, [4] Department of Pathology, Hospital Israelita Albert Einstein, São Paulo, Brazil, [5] Laboratory of Genitourinary Cancer Pathogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, [6] Human Reproduction and Genetics Center, Faculdade de Medicina do ABC, Santo André, Brazil

Background: Prostate cancer (PCa) is a heterogeneous disease that lends itself toward numerous therapeutic options depending on its risk stratification. One of the greatest challenges in PCa urologic practice is to select patients who should be referred for biopsy and, for those patients who are diagnosed with cancer, to differentiate between patients with indolent disease from those with an unfavorable prognosis and, to determine ideal patient management and avoid unnecessary interventions. Accordingly, there is a growing body of literature reporting immunohistochemical studies with the objective of determining a prostate cancer prognosis. Among the most frequent biomarkers studied are Ki-67, p53, PTEN, MYC, and ERG. Based on these findings, we systematically reviewed articles that assessed the role of these main prognostic markers in prostate cancer.

Methods: Consistent with PRISMA guidelines, we performed a systematic literature search throughout the Web of Science and PubMed Medline databases. We considered all types of studies evaluating the role of Ki-67, p53, PTEN, MYC, and ERG immunohistochemical analysis in prostate cancer until July 2017.

Results: We identified 361 articles, 44 of which were summarized in this review. Diagnostically, no single immunohistochemical marker was able to define a tumor as benign or malignant. Prognostically, Ki-67, p53, and MYC were related to the tumor grade given by Gleason score and to the tumor stage (higher levels related to higher tumor grade). Furthermore, Ki-67 was also related to higher PSA levels, shorter disease-free intervals and shorter tumor-specific survival; the latter was also related to p53. The loss of PTEN protein expression showed a higher association with biochemical recurrence and with a worse prognosis, beyond that predicted by the Gleason score and tumor stage. ERG staining also showed a strong association with biochemical recurrence.

Conclusion: There are several studies relating immunohistochemical markers with clinical-laboratorial outcomes in prostate cancer, the most frequent being Ki-67, p53, ERG, PTEN, and MYC. However, none of these markers have been validated by literary consensus to be routinely applied in medical practice.

Keywords: immunohistochemistry, prostate cancer, biomarkers, ERG, MYC, Ki-67, p53, PTEN

INTRODUCTION

Prostate cancer (PCa), excluding non-melanoma skin tumors, is the most prevalent cancer among men (1–3), with adenocarcinoma being the most frequent histological subtype. Despite the controversy (4), prostate-specific antigen (PSA) serum level (1, 3) screening routinely accompanies digital rectal examination. Diagnostic confirmation is accomplished by prostate biopsy guided by transrectal ultrasonography with or without magnetic resonance imaging (MRI) (5).

The heterogeneity of PCa histology was initially described by Donald Gleason in the 1960s and has improved over the years (6). Incorporating modifications to the Gleason grading system, the methodology used today is according to the International Society of Urological Pathology (ISUP) (7).

As a heterogeneous tumor which in turn allows a variety of therapeutic options, depending on its risk stratification, it is extremely important to identify the factors that determine PCa prognosis, thus defining the best course of clinical management. Significant prospective series show that radical treatment does not benefit low-risk patients according to the D'Amico's classification (8–10). Although the number of patients diagnosed with low-risk tumors and subjected to unnecessary surgery is substantial (11, 12).

Clinicopathological nomograms are commonly used to stratify risk, although with technological development and an increased understanding of tumor biology, immunohistochemical (IHC) and molecular biomarkers are emerging as powerful tools to distinguish tumors with distinct behaviors.

Immunohistochemical analysis consists of using monoclonal or polyclonal antibodies to detect specific antigens in tissue samples, and it is a widely used technique that can be applied in diverse situations, such as cellular differentiation, characterization of a tumor's primary site, detection of metastases, prognostic factors, as a predictor of targeted therapy response and even in the identification of structures, organisms and materials secreted by cells of interest. In prostate cancer, immunohistochemistry has an important role in the diagnostic confirmation of borderline cases due to the presence (or absence) of basal cells, detected by specific antibodies against it combined with racemase expression in luminal epithelial cells.

Currently, the identification of biomarkers capable of predicting the course of the disease has been gaining importance (12–14).

Among the most frequent biomarkers studied that are associated with PCa are p53 (tumor protein p53), Ki-67 (marker of proliferation), ERG (ETS-related gene), MYC (proto-oncogene), and PTEN (phosphatase and tensin homolog), which are implicated in the control of cell proliferation and differentiation, angiogenesis, and apoptosis (15–25).

In the main series of active surveillance, approximately 30% of patients are reclassified and subjected to radical therapy. Immunohistochemical markers could be useful for the initial evaluation and for identifying cases with major or minor potential of progression, helping the decision process of whether to start a radical treatment. Another important application would be in patients with a low volume of ISUP 2, for which immunohistochemistry (IHQ) could be useful in selecting candidates for active surveillance.

Based on these findings, we systematically reviewed articles that studied the role of these main IHC prognostic markers in prostate cancer.

METHODS

The systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (26).

Search Strategy

A computerized literature search of PubMed to identify title and abstracts published was performed. The search was performed with and without MESH terms (Ki-67, p53, PTEN, MYC, ERG, immunohistochemistry, and prostate cancer). All references in the selected articles were checked, including hand-typed searches.

Study Eligibility

The final articles were selected based on the following set of inclusion criteria: (i) examined the association of immunohistochemical expression of Ki-67, p53, PTEN, MYC, and/or ERG with clinical outcome of prostate cancer; (ii) in humans; (iii) non-metastatic prostate cancer; (iv) articles published from 1966 until July 2017. No restriction was made regarding the study type or language. Articles were excluded for any of the following reasons: (i) could not be accessed in its entirety; (ii) were duplicated; (iii) were a review article.

Initially, titles were reviewed to assess whether they met the inclusion criteria. If, after assessing the abstract, there was any doubt regarding whether it met the relevant criteria, it was kept for more thorough, subsequent assessment. The list of potential

Abbreviations: PCa, Prostate cancer; PSA, Prostate-specific antigen; ISUP, International Society of Urological Pathology; IHC, Immunohistochemical; ERG, ETS-related gene; PTEN, Phosphatase and tensin homolog; TUR, Transurethral resection; MRI, Magnetic resonance imaging; EAU, European Association of Urology; AUA, American Urological Association.

articles was further shortened by performing detailed evaluations of the methods and results of each remaining paper.

We included patients with localized or locally advanced prostate cancer submitted for or in the process of definitive treatment. None of these patients were submitted to radiotherapy or hormonal therapy before biopsy or prostatectomy.

Data Collection

The following details were recorded for each study: author, year of publication, country where study was performed, study design, number of patients, population/setting, type of material used, main objective of the study (diagnostic and prognostic evaluation) and outcome reported (Gleason Score, PSA level, tumor stage, lymph node stage, tumor diameter, and Gleason upgrading).

RESULTS

The literature search identified a total of 361 studies. Using the above inclusion and exclusion criteria a total of 42 studies were included in the systematic review: 9 concerning Ki-67 and p53; 3 concerning MYC; 15 concerning PTEN, and 17 concerning ERG. (2 studies concerned more than one marker). A schematic of the search is depicted in **Figure 1**.

The attachment archive depicts the characteristics of the studies included in systematic review based on the association of the immunohistochemical expression of Ki-67, p53, PTEN, MYC, and/or ERG with the clinical outcomes of prostate cancer (**Supplementary Table 1**).

Table 1 lists the main outcomes and studies, according to the studies that analyzed biopsy specimens and prostatectomy specimens.

Diagnosis

The vast majority of prostate cancer diagnoses are based on morphological assessment. IHC is complimentary in case of doubt, but its interpretation should always be evaluated in conjunction with histomorphology. None of the IHQ markers used with a diagnostic purpose (p63, high molecular weight keratins, racemase and ERG) are specific and/or sensitive enough to classify a case as benign or malignant.

Prognosis

Ki-67

Ki-67 is a protein expressed in the nucleus of proliferating cells during mitosis interphase. It is expressed during late G1, S, G2, and M phases, but not during G0 phase (15). The Ki-67 protein is
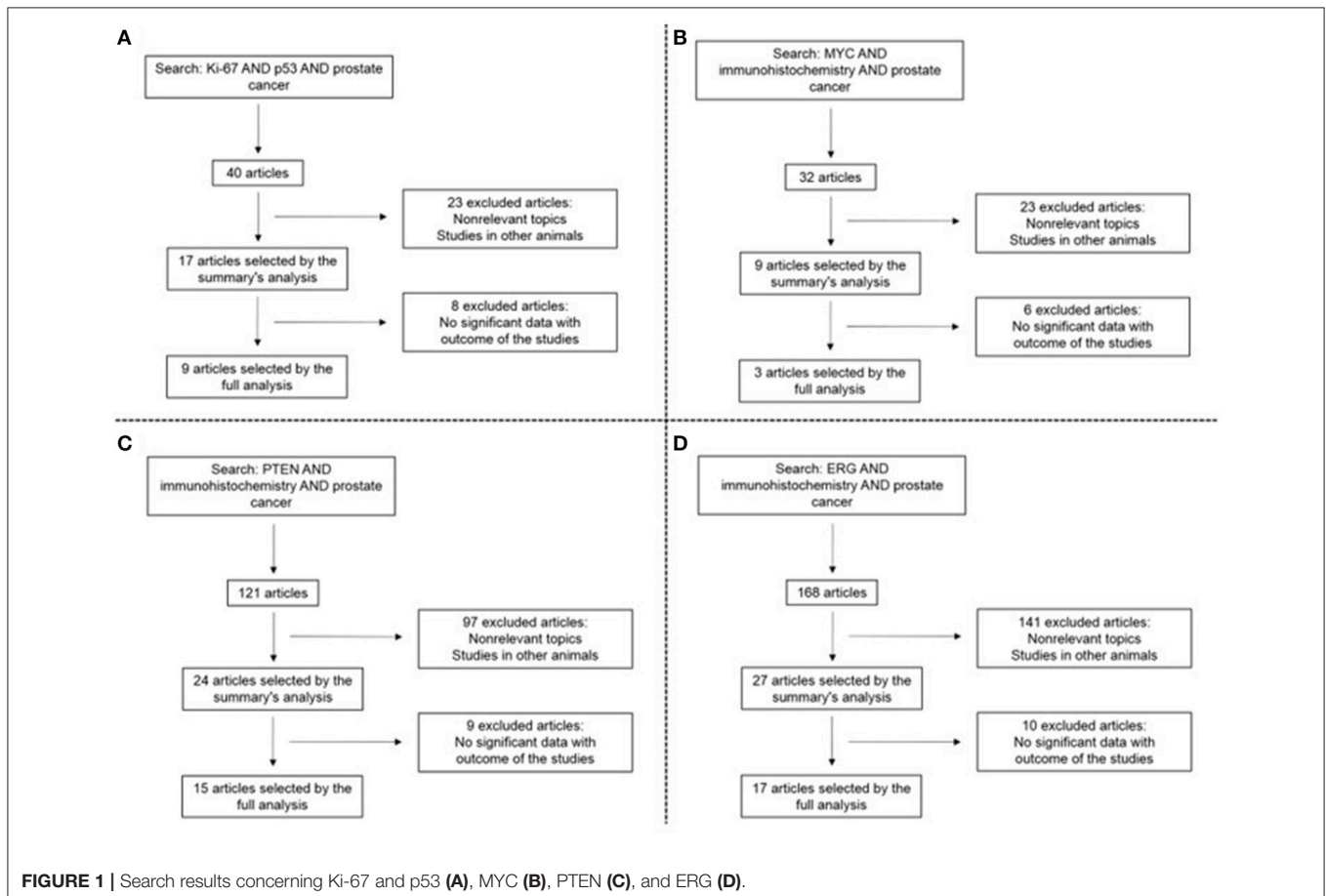

FIGURE 1 | Search results concerning Ki-67 and p53 **(A)**, MYC **(B)**, PTEN **(C)**, and ERG **(D)**.

TABLE 1 | Immunohistochemical marker role in predicting outcomes in biopsy and surgical specimen analysis.

	ERG	PTEN	MYC	Ki-67	p53
BIOPSY SPECIMEN					
Gleason score	~	✓(27–29)	~	✓(30, 31)	✓(31, 32)
PSA level	✓(33)	~	~	✓(30)	✓(30)
T-stage	~	✓(27, 34, 35)	✓(36)	✓(32)	✓(32)
N-stage	~	~	~	✓(32)	~
Clinical insignificance	~	✓(28)	~	✓(30)	~
Tumor diameter	~	~	~	~	~
Gleason upgrading	~	✓(35, 37)	~	✓(32)	~
Disease-free interval	~	✓(28, 35, 38, 39)	~	~	~
Overall survival	~	~	✓(36)	~	~
Tumor-specific survival	~	~	~	✓(31)	✓(31)
SURGICAL SPECIMEN					
Gleason score	✓(40–45)	✓(28, 46–49)	✓(50)	✓(51–53)	✓(32, 51, 52, 54)
PSA level	✓(33, 40, 44, 45, 55, 56)	✓(48)	~	~	~
T-stage	✓(25, 57–59)	✓(34, 46–49, 60)	✓(25, 36, 50)	✓(32, 51–53)	✓(32)
N-stage	~	✓(48)	~	✓(32, 51)	~
Clinical insignificance	~	✓(28)	~	~	~
Tumor diameter	~	~	~	~	~
Gleason upgrading	~	~	~	✓(32)	~
Disease-free interval	✓(40, 57, 61–63)	✓(28, 38, 46, 47, 49, 60, 64)	~	✓(32, 53, 65)	✓(65, 66)
Overall survival	✓(43)	✓(48)	✓(36)	✓(53)	✓(53)
Tumor-specific survival	✓(38)	✓(48)	~	✓(53)	✓(53)

✓, Studies showed correlation; ~, No studies were found with this analysis.
(n), Studies in accordance to the references.

a cellular proliferation marker (16, 17) and it is widely used given its high reproducibility (15).

Of the selected articles, the analyzed materials in studies were from: prostatectomy specimens (5 studies) (32, 51–53, 65), biopsy specimens (3 studies) (30–32), and transurethral resections (TUR) (2 studies) (52, 53).

Chevrier et al. (51) quantitatively and positively correlated Ki-67 expression based on the H-score and tumor grade given by the Gleason score: H-score 2, 21, and 52 for Gleason score ($3 + 3 = 6$, $3 + 4 = 7$, and $4 + 4 = 8$), respectively ($p = 0.0002$). Other studies also showed significant statistical correlation between Ki-67 and the Gleason score (30, 31, 52, 53), of which 2 were biopsy specimens (30, 31) and the others were prostatectomy and/or TUR specimens (52, 53).

Ki-67 protein overexpression was also shown to be related to T stage and lymph node invasion (32, 51). Its higher expression was more related to stage T3 than to T2 ($p = 0.0001$), and found more in N1 ($p = 0.0389$) the latter also shown in Zellweger et al. (32). Zellweger et al. (32) showed that Ki-67 >10% (high) was associated with adverse pT stage ($p = 0.036$), specifically to seminal vesicle invasion (pT3b) with OR = 12 (2.5–57.0, 95% CI), $p = 0.002$.

Elevated Ki-67 showed an association with PSA serum levels for PSA ≤ 2.5 ng/ml compared with PSA between 10 and 20 ng/ml ($p < 0.05$) (30). In turn, low levels of Ki-67 were related to clinically insignificant tumors [determined by the

Epstein criteria (67) and by PRIAS (68)], at $p = 0.05$ and 0.005, respectively (30).

In a study comparing biopsy and prostatectomy specimens, an elevated Ki-67 (>10%) in the biopsy was related to a higher Gleason score in the prostatectomy specimen ($p = 0.001$) (32). In the same study, an association between Ki-67 and the disease/biochemical recurrence-free interval was found, as also shown by Inoue et al. (65).

In general, an association between elevated Ki-67 and a higher percentage of tumor cell involvement on biopsy ($p = 0.037$), as well as shorter tumor-specific survival ($p = 0.0007$), can be found in the literature (31).

p53

Tumor protein 53 (p53) regulates the control of cell cycle progression and cell proliferation, as well as the intrinsic mitochondrial apoptosis pathway (16, 18). p53 acts as a transcription factor, controlling genes to prevent cell proliferation after DNA damage (19). When mutations in the *TP53* gene occur, cells that contain damaged DNA are not repaired nor are cells destroyed, resulting in the inability to stop the cell cycle or to initiate apoptosis, thus allowing the emergence of malignant cells (20). Mutations in *TP53* occur in more than 50% of malignancies, including high grade prostate cancers (21).

Of the selected articles, the analyzed materials in the studies were: specimens from prostatectomies (7 studies) (32, 51–54, 65, 66), biopsies (3 studies) (30–32), and TUR (2 studies) (52, 53).

Regarding p53 as an immunohistochemical marker, the relationship between its expression and tumor grade (Gleason score) was reported in various studies (32, 51, 52, 54). Chevrier et al. (51) showed this relationship in a quantitative manner using the H-score system, comparing histologic groups as follows: Gleason 3 + 3 had an H-score of 3, Gleason 3 + 4 had an H-score of 65 and Gleason 5 + 4 H-score = 195 ($p = 0.0430$) (51).

Zellweger et al. (32) demonstrated the relationship between p53 positivity (>20% of highly reactive nuclei) and seminal vesicle invasion ($p = 0.001$) (32). Nagao et al. showed a relationship between p53 and the PSA level (comparing PSA$\leq$2.5 ng/ml and PSA between 10 and 20 ng/ml) ($p < 0.05$) (30).

An association between p53 and biochemical recurrence free survival was shown in two studies, Inoue et al. (65) and Osman et al. (66) ($p = 0.0097$ and $p < 0.01$, respectively) (65, 66). In survival studies, p53 overexpression predicted shorter tumor-specific survival ($p < 0.05$) (53) and overall survival ($p = 0.024$) (31).

ERG

ERG (ETS-related gene) expresses an essential protein for the maintenance of vascular integrity. It is associated with cellular structure maintenance, and its loss leads to higher endothelial permeability. It is also associated with haematopoiesis, maintenance of platelets in peripheral blood and pre-cartilage formation (in mesoderm cells). Typically, it is not present in normal prostatic tissue. The protein is overexpressed in prostate cancer when the androgen-induced *TMPRSS2 (transmembrane serine protease 2)* gene fuses to the *ERG* gene (22).

Of the selected articles, the analyzed materials in the studies were specimens from prostatectomies (17 studies) (25, 33, 38, 40–45, 55–59, 61–63), TUR (3 studies) (33, 38, 59), biopsies (2 studies) (33, 38).

In the analysis of reviewed articles, there was a consistency between the relationship of ERG protein expression and the biochemical recurrence of PCa (38, 40, 57, 61–63). Hagen et al. showed that 13 of 28 patients (46.4%) who had surgical specimens with high ERG levels developed recurrence compared to only 3 (12%) of 25 patients who had tumors with low ERG levels ($p = 0.006$) (57). Font-Tello et al. also showed this association, with PSA progression observed in 3 of 25 (12%) ERG-negative patients compared to 13 of 38 (34.2%) ERG-positive patients ($p = 0.04$) (61). Kim et al. presented a relative risk for biochemical recurrence in ERG-positive patients of 8.964 ($p = 0.002$) (40).

An association between ERG-positive immunohistochemistry and Gleason score was frequently reported (41–45). Suh et al. (41) showed that ERG protein expression was more frequently detected in the subgroup with a lower primary Gleason grade (less than or equal to 7) than in the subgroup with a higher Gleason grade ($p = 0.011$) (41). Kron et al. (42) obtained similar results, with ERG positivity found more frequently in Gleason score 6 and 7 tumors, whereas a Gleason score of 8 to 10 displayed a lower positive frequency ($p < 0.01$) (42).

For PSA levels, ERG-positive cases were associated with lower preoperative PSA compared to ERG-negative cases (33, 40, 44, 45, 55, 56). Brooks et al. (45) showed a mean PSA of 7.9 ng/ml in patients with positive ERG expression and a mean PSA of 9.3 ng/ml in ERG-negative patients ($p = 0.0003$) (45). Kim et al. compared a group of patients with PSA serum levels lower than 10 ng/ml to a group of patients with PSA higher or equal to 10 ng/ml and showed that the relative risk for positive ERG protein expression was 4.3 favorable for the group with lower PSA levels ($p = 0.039$) (40).

For the presence of adverse pathologic features, Udager et al. (25) showed that ERG-positive tumors were associated with extraprostatic extension ($p = 0.02$). They also showed that these tumors were associated with elevated pathological stage greater than or equal to pT3 ($p = 0.035$) (25). This correlation was also reported by other groups (58, 59, 61).

Finally, several reports indicated that ERG-positive patients were enriched for PCa diagnosis at a younger age (44, 45, 55, 59). Schaefer et al. showed a mean age of 60 years for ERG-positive tumors in contrast to 63 years for ERG-negative tumors ($p < 0.0001$)(44). Similarly, Brooks et al. showed a mean age of 60.5 vs. 62.5; $p < 0.0001$, with the ERG-positive expression group having a younger age (45).

PTEN

PTEN (phosphatase and tensin homolog) is a tumor suppressor gene. It is a lipid phosphatase and a negative regulator of the PI3K/AKT/mTOR pathway, which controls cellular processes such as survival, proliferation, metabolim, migration and cellular architecture. The PTEN protein is frequently absent in some cases of prostate cancer, indicating a loss of function (23).

Of the selected articles, the analyzed materials in the studies were: specimens from prostatectomies (10 studies) (27, 28, 34, 38, 46–49, 60, 64), biopsies (6 studies) (28, 29, 35, 37–39), and TUR (3 studies) (34, 38, 69).

One of the most consistent correlations found in the literature was between the loss of PTEN and a higher recurrence rate or shorter disease-free interval, with the finding that PTEN is considered a biochemical recurrence predictor (28, 34, 38, 39, 46, 47, 60, 64). Murphy et al. showed that among Gleason score 7 or higher tumors, those with PTEN loss had a recurrence rate of 80% compared to 55% in those with intact PTEN (28).

In addition, a correlation between loss of PTEN staining and a worse disease prognosis was frequently observed. Studies revealed a higher risk of death among patients with prostate cancer and loss of PTEN staining compared to patients with proficient and/or partially reduced PTEN protein staining (48, 49, 69). Lahdensuo et al. presented a hazard ratio for risk of death by prostate cancer of 2.156 (95% CI 1.169–3.976, $p = 0.014$) in a univariate analysis that compared those with a total loss of PTEN to those with partial or no loss of PTEN (48).

Lotan et al. reported a relationship between the loss of PTEN protein and Gleason upgrading from biopsy to prostatectomy. They found that 18.3% (13/71) of tumors that had Gleason upgrading presented PTEN protein loss compared to 7% (7/103) of those without Gleason upgrading ($p = 0.02$) (37). Guedes et al. also showed this correlation (35).

PTEN loss was associated with extraprostatic extension in numerous studies (35, 46, 47), notably seminal vesicle invasion

(46, 47, 60). It also showed an association with a higher Gleason score (27, 29, 46–49). Genomically, homozygous loss of *PTEN* was present in only 4% of patients with Gleason score 6 compared to 18% of those with a Gleason score of 8 to 10 ($p < 0.0001$) (47).

MYC

MYC is a nuclear transcription factor related to regulation of the cell cycle progression, metabolism, ribosome biogenesis, protein synthesis, mitochondrial functions and stem cell self-renewal. Its protein is frequently overexpressed in prostate cancer and can have a role in tumor initiation and/or progression (24, 25).

Of the selected articles, the analyzed materials in the studies were specimens from prostatectomy (3 studies) (25, 36, 50) and biopsies (2 studies) (25, 36).

In our literature review, we found correlations between MYC and the Gleason score. Prowatke et al. (50) showed that a decrease of MYC protein expression was related to an increase in Gleason score. MYC expression was 74, 54, and 28% in tumors with Gleason score 6, 7 and 8–9, respectively ($p = 0.001$) (50).

A correlation between a reduction of MYC protein expression and an increase of T-stage was also demonstrated, with 73% in pT2 and 36% in pT3–4, ($p = 0.001$) (50). These findings were also reported by Zeng et al. (36) ($p < 0.001$). Udager et al. demonstrated that MYC overexpression was related to the presence of extraprostatic extension ($p = 0.004$) in a multivariate analysis with HR= 5.780 (95% CI 2.125 to 15.722) ($p < 0.001$) (25).

DISCUSSION

Prostate cancer screening is constantly questioned because large studies failed to demonstrate any survival benefit (70–72) and because the overdiagnosis caused by the detection of indolent tumors leads to overtreatment and a worsened quality of life because of the treatment (73).

Although active surveillance should remain the preferred option in managing very low-risk and the majority of low-risk prostate cancers (74), it is constantly under-used in clinical practice worldwide. According to the US National Cancer Data Base, in 2013, less than 20% of men with low-risk prostate cancer were managed conservatively (75).

Active surveillance presents a cancer-specific survival similar to active treatment in very low and low-risk PCa patients. However, the disease progression-free survival rate is significantly higher in active surveillance patients, compared to men treated with radical prostatectomy or radiation therapy (9). Therefore, it is of foremost importance to determine whether there are any other factors that could improve the selection of patients that can be safely managed without definitive treatment.

For this, several IHC markers, are readouts for tumor behavior and were used to better define the prognosis and course of management for men with prostate cancer. However, there remains no literary consensus about which markers are more

reliable for those purposes. In this manuscript, we emphasized what we believe to be the most promising markers, p53, Ki-67, MYC, ERG and PTEN, analyzed in both the biopsy and post-prostatectomy setting.

In the diagnostic scenario, in this systematic review, no immunohistochemical marker was found to be significant for diagnosing or defining a tumor as benign or malignant.

By contrast, for determining prognosis, immunohistochemical markers stand out in several studies, some presenting with consistent results. Ki-67, p53, and MYC were consistently related to the tumor grade given by Gleason score and to the tumor stage (higher levels related to higher tumor grade). Ki-67 was also related to higher PSA levels, shorter disease-free interval and shorter tumor-specific survival. Additionally, data showed a relationship between p53 and shorter tumor-specific survival. The loss of PTEN protein expression was related to biochemical recurrence and a worse prognosis, beyond Gleason score and tumor stage. One of the most consistent findings was the association between ERG staining and biochemical recurrence.

Considering all these findings in this systematic review, we propose that immunohistochemical markers are in the process of becoming consistent prognostics tools in clinical practice. As a scientific approach, the relationship between these markers and certain outcomes in prostate cancer is increasingly shown. However, gaining statistical significance and strength in clinical practice remains necessary.

Although cost-effective analyses are missing, a wider utilization of IHC markers in daily routine practice could facilitate decision-making by the clinician and even encourage the patient to follow the most appropriate path for disease management.

CONCLUSION

In summary, there are several studies relating immunohistochemical markers with clinical-laboratory outcomes in prostate cancer, the most frequent being Ki-67, p53, ERG, PTEN, and MYC. However, none of these markers have been validated and, consequently, they cannot be applied in medical practice.

Positive staining for Ki-67, p53 and MYC were related to higher tumor grade and stage. Ki-67 was also related to PSA levels, disease-free interval and tumor-specific survival (the latter also being related to p53). For PTEN, its loss showed a higher association with biochemical recurrence and a worse prognosis, as well as Gleason score and tumor stage. Finally, ERG showed a strong association with biochemical recurrence.

If applied in specific situations, the use of these markers could guide the process of therapeutic decision making.

AUTHOR CONTRIBUTIONS

AC, ÁB MW and BB drafted the manuscript and worked on the conception, design and interpretation of data. ÁB and LT selected articles, screened titles and abstracts, assessed study quality and

extracted data. AC, ÁB, LT, PK, NM, CC, MW, IC, BB, GL, AS, MO and MT-M were involved in the interpretation and discussion of the results and critically revised the systematic review for important intellectual content. All authors approved the final version of the systematic review.

SUPPLEMENTARY MATERIAL

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