RESEARCH ARTICLE

Prognostic Factors of Prostate Cancer in Tunisian Men: Immunohistochemical Study

Nabiha Missaoui[1,2]*, Soumaya Ben Abdelkarim[3], Moncef Mokni[3], Sihem Hmissa[1,3]

Abstract

    <u>Background</u>: Prostate cancer is the second most common male cancer and remains a leading cause of cancer death worldwide. Heterogeneity regarding recurrence, tumor progression and therapeutic response reflects the inadequacy of traditional prognostic factors and underlies interest in new genetic and molecular markers. In this work, we studied the prognostic value of the expression of 9 proteins, Ki-67, p53, Bcl-2, PSA, HER2, E-cadherin, p21$^{WAF1/Cip1}$, p27^{Kip1} and p16^{ink4a} in prostate cancer. <u>Materials and Methods</u>: We conducted a retrospective study of 50 prostate cancers diagnosed in Pathology Department of Farhet Hached Hospital, Sousse, Tunisia, during a period of 12 months. Clinico-pathological data and survival were investigated. Protein expression was analyzed by immunohistochemistry on archived material. <u>Results</u>: Expression or over-expression of Ki-67, p53, Bcl-2, PSA, HER2, E-Cadherin, p21$^{WAF1/Cip1}$, p27^{Kip1} and p16^{ink4a} was observed in 68%, 24%, 32%, 78%, 12%, 90%, 20%, 44% and 56% of cases, respectively. Overall five-year survival was 68%. A statistically significant correlation was observed between death occurrence and advanced age (p=0.018), degree of tumor differentiation (p=0.0001), perineural invasion (p=0.016) and metastasis occurrence (p=0.05). Death occurrence was significantly correlated with the expression of p53 (p=0.007), Bcl-2 (p=0.02), Ki-67 (p=0.05) and p27^{Kip1} (p=0.04). <u>Conclusions</u>: The p53, Bcl-2, Ki-67 and p27^{Kip1} proteins may be useful additional prognostic markers for prostate cancer. The use of these proteins in clinical practice can improve prognosis prediction, disease screening and treatment response of prostatic cancer.

Keywords: Prostate cancer - prognosis - markers - immunohistochemistry

Introduction

    Prostate cancer is a major public health problem worldwide. It represents the second most common male cancer after lung cancer (Globocan, 2012; Daniyal et al., 2014; Van Dong et al., 2014; Baade et al., 2015; Bashir, 2015). In 2012, approximately 1.1 million prostate cancers were diagnosed representing 15% of male cancers and 70% of cases were recorded in developed countries (Globocan, 2012). Prostate cancer remains a leading cause of male cancer-related deaths worldwide with about 307 000 deaths in 2012 (Globocan, 2012). In Tunisia, it ranks third after lung and bladder cancers with an incidence rate of 11.6/100,000 (Missaoui et al., 2010). At present, there is no large screening program for prostate cancer. The prostate-specific antigen (PSA). testing is done only in university institutions (Missaoui et al., 2010).

    Prostate cancer is a heterogeneous pathology and displays a range of clinical behavior, from a slow-growing tumor of no clinical significance to aggressively metastatic and lethal disease. The basis of this variable prognosis is poorly understood despite considerable research. The use

of PSA testing has led to the diagnosis of many potentially indolent cancers, and aggressive treatment of these cancers has caused significant morbidity without clinical benefit in many cases. In order to improve therapeutic strategies in patients with advanced disease and to avoid over treatment or under treatment of patients, it has become necessary to establish new prognostic factors for prostate cancer. The major problem of this cancer lies in its evolutionary whims. The heterogeneity regarding recurrence, tumor progression and therapeutic response for tumors having the same prognostic criteria at diagnosis reflects the inadequacy of traditional factors used and the need for new molecular and genetic markers (Huges et al., 2005; Quinn et al., 2005; Bensalah et al., 2008; Molinié et al., 2008; Ramirez et al., 2008; Wang et al., 2012; Bostrom et al., 2015; Esfahani et al., 2015).

    For many years, new tissue prognostic factors have been described (Huges et al., 2005; Quinn et al., 2005; Bensalah et al., 2008; Wang et al., 2012). Recent studies have emphasized the importance of several markers of cell proliferation and apoptosis, tumor suppressor genes and adhesion molecules in assessing the prognosis of prostate

[1]Research Unit UR14ES17, Cancer Epidemiology and Cytopathology in Tunisian Center, Medecine Faculty of Sousse, University of Sousse, [2]Faculty of Sciences and Technologies of Sidi Bouzid, Kairouan University Tunisia, [3]Pathology Department, Farhet Hached Hospital, Sousse, Tunisia *For correspondence: missaouinabiha@live.fr

cancer. Incorporation of these markers into clinical practice has the potential to improve disease screening, prognostic discrimination and prediction of response to treatment (Huges et al., 2005; Quinn et al., 2005; Bensalah et al., 2008; Molinié et al., 2008; Ramirez et al., 2008; Wang et al., 2012; Bostrom et al., 2015; Esfahani et al., 2015). In this context, we studied the expression of 9 genes, including Ki-67, Bcl-2, PSA, p53, HER2, E-cadhérine, p16^{ink4a}, p21$^{WAF1/Cip1}$ and p27^{Kip1} in prostate cancer in Tunisian men.

The Ki-67 is a cell proliferation marker. p53 and Bcl-2 proteins are involved in the progress of cell cycle control and the apoptosis regulation. The PSA is a protease secreted by the epithelial cells of the acini and ducts of prostate tissue. The HER2 is the receptor 2 of human epidermal growth factor. E-Cadherin is a cell-adhesion protein. The cyclin-dependent kinase (CDK). inhibitory genes involved in cell cycle progression include p21$^{WAF1/Cip1}$ and p27^{Kip1}, who are Cip/Kip family inhibitors, and p16^{ink4a} which belongs to the INK4A family inhibitors and inhibits the formation of cyclin D/CDK4, 6 complex.

In this study, we analyzed the correlation between the expression of Ki-67, p53, Bcl-2, PSA, HER2, E-cadherin, p21$^{WAF1/Cip1}$, p27^{Kip1} and p16^{ink4a} proteins and the tumor evolving profile and the occurrence death in order to assess the utility of the expression of these proteins as specific prognostic marker for prostate cancer in Tunisian men.

Materials and Methods

Tissue samples

We carried out a retrospective study of 50 prostate cancers retrieved from the files of Urology Department, Sahloul University Hospital and Radiotherapy and Pathology Departments, Farhet Hached University Hospital, Sousse (Tunisia). for a period of 12 months (January 2007-December 2007). The diagnosis was established in the Pathology Department, Farhet Hached University Hospital of Sousse.

The material concerned endoscopic resection, prostate punctures or biopsies, pieces of transvesical adenomectomy and radical prostatectomy. All tissues had been routinely fixed in 4% buffered formalin and paraffin embedded and slides were reviewed by two pathologists (Pr. Hmissa and Dr. Ben Abdelkarim).

One or two paraffin blocks containing representative portions of the tumors were selected for each case, and 4-μm-thick sections were obtained.

The study was approved by the Human Ethics Committee at the Farhet Hached University Hospital of Sousse (Tunisia). and it conformed to the provisions of the Declaration of Helsinki.

Clinico-pathological study

The collection of clinico-pathological data was conducted using patient clinical records from Urology Department, Sahloul University Hospital, Radiotherapy and Pathology Departments, Farhet Hached University Hospital, Sousse.

Age at diagnosis, cancer discovery circumstances, PSA level at the time of diagnosis, type of prostate removal, macroscopic features, tumor size, histological type, perineural invasion, tumor stage, Gleason score, degree of tumor differentiation, the onset of metastasis, progression and survival were recorded.

Immunohistochemistry

The expression of 9 genes including Ki-67, Bcl-2, PSA, p53, HER2, E-Cadherin, p16^{ink4a}, p21$^{WAF1/Cip1}$ and p27^{Kip1} was performed by immunohistochemistry in the Pathology Department, Farhet Hached University Hospital, Sousse. Formalin-fixed, paraffin-embedded tissues were deparaffinized in xylene, rehydrated through serial dilutions of alcohol and washed in phosphate–buffered saline (ph7.2). After pretreatment with antigen retrieval solution in 10 mM citrate buffer at 95°C for 40 minutes in a streamer, endogenous peroxidase activity was blocked in 3% hydrogen peroxide. The slides were incubated with the primary antibody at room temperature (Table 1). The revelation was made by the Envision Kit + Dual Link System HRP (Dako, code K4063). Diaminobenzidine was used as the chromogen for the immunostaining. Finally, sections were counterstained with hematoxylin and mounted. Specific positive controls were used for each antibody. Negative controls were obtained by excluding the primary antibody. The images were captured by the Olympus microscopic digital camera system for study comparison.

Immunohistochemistry interpretation

Immunohistochemical staining was estimated by two independent pathologists (Pr. Hmissa and Dr. Ben Abdelkarim). For HER2 immunostaining, only membrane staining was evaluated. We adopted the 2007 guideline recommendations of the American Society of Clinical Oncology and the College of American Pathologists (ASCO-CAP) (Wolff et al., 2013). Therefore, "positive" is defined as strong, complete, homogenous membrane staining in >30% of tumor cells, "equivocal" is defined as either strong, complete, homogeneous membrane staining in <30% or weak, moderate heterogeneous complete membrane staining in >10% of tumor cells, and "negative" is defined as no staining, or weak, incomplete membrane staining in any percentage of cells.

For other antibodies, the immunostaining evaluation was made quantitatively on 400 nuclei with calculation of the labeled cells number divided by the cells counted number. The immunostaining was considered either positive or negative. The percentage of tumor cells was indicated in all positive cases.

Statistical analysis

Statistical analyzes were performed using SPSS software Version 20.0. The Chi-square and the Fisher-exact tests were used. Survival was calculated using the Kaplan-Meier method. The Log-Rank test was used to compare survival curves. The correlation is considered significant for a probability greater than 95%. Probability values of 0.05 or less were considered statistically significant.

Results

Clinico-pathological features

The patient median age was 73.4 years (55-93 years). and 96% of patients were older than 60 years. The majority of patients were symptomatic with obstructive type signs such as dysuria (84%). and irritative type signs such as pollakiuria (78%). Only in 4% of cases, the tumor discovery resulted from a screening protocol. In the majority of patients (84.8%)., PSA level was greater than 10 ng/ml with a mean of 258.9 ng/ml.

According to Gleason score, 42% of prostate adenocarcinomas had a score ≤ 6 and 28% of cases had a score ≥ 8. The perineural invasion was found in 22% of cases. Carcinomatous lymphangitis and venous invasion were absent in all cases of our series. The prostatic

adenocarcinoma was moderately differentiated in 68% of cases and poorly differentiated in 28% of cases. Only two tumors were well-differentiated.

Immunohistochemistry

The expression of PSA was observed in 78% of tumor cases. This expression was cytoplasmic and ranged from 30 to 100% of the tumor cells (Figure 1A). Only 12 prostate adenocarcinomas expressed the p53 protein (24%, Figure 1B). The Ki-67 expression was observed in 68% of prostate cancers. The expression levels ranged from 1 to 70% of tumor cells (Figure 1C). The majority of tumors expressed E-Cadherin (90%). reaching 70 to 100% of tumor cells (Figure 1D). Sixteen cancers expressed Bcl-2 with an expression rate of 2 to 100% of tumor cells (32%, Figure 1E). HER2 expression was observed in 12% of prostate adenocarcinomas (Figure 1F).

Cip/Kip family of CDK inhibitors: $p21^{WAF1/Cip1}$ and $p27^{Kip1}$ were expressed in 20% and 44% of prostatic adenocarcinomas, respectively (Figure 1G, 1H). $p16^{ink4a}$ protein was expressed in 56% of tumors with a nuclear staining reaching 2 to 100% of tumor cells (Figure 1I).

In this study, no significant correlation was observed between the expression of Ki-67, Bcl-2, PSA, p53, HER2, E-Cadherin, $p16^{ink4a}$, $p21^{WAF1/Cip1}$ and $p27^{Kip1}$ and the age at the diagnosis, the Gleason score, and the PSA level.
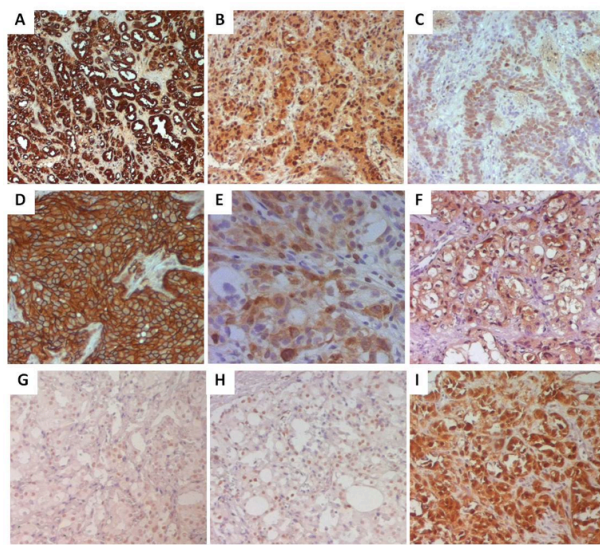

Figure 1. Expression of PSA, p53, Ki-67, E-Cadherin, Bcl-2, HER2, p21WAF1/Cip1, p27Kip1 and p16ink4a in prostate cancer: (A) strong and diffuse PSA expression [Mx40]; (B) strong nuclear p53 expression [Mx100]; (C) focal nuclear Ki-67 expression [Mx100]; (D) strong membrane E-Cadherin expression [Mx100]; (E) nuclear Bcl-2 expression [Mx200]; (F) positive HER2 expression [Mx100]; (G) focal nuclear p21WAF1/Cip1 expression [Mx100]; (H) focal nuclear p27Kip1 expression [Mx100]; (I) strong nuclear p16ink4a expression [Mx100].
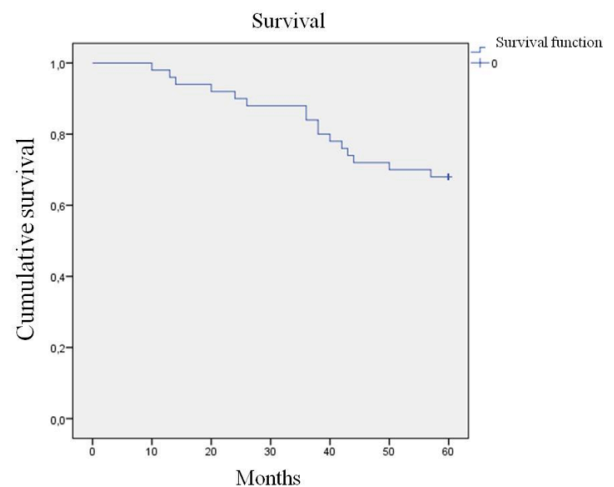

Figure 2. Overall survival in patients with prostate cancer

Table 1. Antibodies and details of immunohistochemical protocols

Antibodies	Supplier (clone)	Dilution	Citrate solution	Staining
Ki-67	Dako (MIB-1 Clone)	1/50th	pH = 6	Nuclear
PSA	Dako	1/25th	pH = 6	Cytoplasmic
HER2	HerceptTest kit, Dako	1/350th	pH = 6	Membrane
E-Cadherin	Leica Biosystem (36B5 Clone)	1/50th	pH = 9	Membrane
p53	Dako (DO-7 Clone)	1/50th	pH = 6	Nuclear
Bcl-2	Dako	01/50th	pH = 6	Nuclear
p16ink4a	CINtec ® p16 Histology Kit, Ventana	Ready for use	pH = 6	Nuclear
p21WAF1/Cip1	Dako (SX118 Clone)	1/25th	pH = 6	Nuclear
p27Kip1	Dako (SX53G8 Clone)	1/25th	pH = 9	Nuclear

Survival

In our study, 16 patients were reported died within 1 month to 5 years. Overall survival was 98% at 1 year and 68% at 5 years (Figure 2). We found a statistically significant correlation between the death occurrence and the advanced age (p=0.018), the tumor differentiation degree (p=0.0001), the perineural invasion (p=0.016). and the metastases occurrence (p=0.05). The onset of death was significantly correlated with the expression of the p53 (p=0.007)., Bcl-2 (p=0.02)., Ki-67 (p=0.05). and p27Kip1 (p=0.04).

Discussion

Prostate cancer is the most common urologic malignancy characterized by the highly variable behavior and clinical course and the diversity of treatment options (Daniyal et al., 2014; Van Dong et al., 2014; Baade et al., 2015; Bashir, 2015). Several research approaches are interested to find prognostic markers for prostate cancer in order to improve the prediction of patient outcomes and therapeutic responses. In the present study, we confirmed the prognostic value of clinico-pathological parameters such as advanced age at diagnosis, the poorly differentiated tumors, the perineural invasion and the occurrence of bone metastasis in prostate cancer in Tunisian men (Yamashita et al., 1993; Maru et al., 2001; Molinié et al., 2008; Salomon et al., 2010). However, the heterogeneity regarding recurrence, tumor progression and therapeutic response reflects that the classical prognostic factors appear to be insufficient to predict prostate cancer evolution. The use of new molecular and genetic markers could improve the prediction of the prostate cancer evolution and the guiding choice of therapy (Huges et al., 2005; Quinn et al., 2005; Bensalah et al., 2008; Molinié et al., 2008; Ramirez et al., 2008; Wang et al., 2012; Bostrom et al., 2015; Esfahani et al., 2015).

p53 and Bcl-2 proteins are the major regulators of mitochondrial or intrinsic apoptotic pathway. The expression level of p53 is heterogeneous in prostate cancer and it ranged from 12% to 97% (Quinn et al., 2005). It was 24% in our study. Most series have observed p53 overexpression in high-grade prostate tumors (Huges et al., 2005; Quinn et al., 2005). The expression of p53 has an important value in evaluating the long-term prognosis of prostate cancer. Indeed, the frequency of p53 expression is significantly correlated with the tumor stage, the hormone escape, the occurrence of bone metastases, the cancer mortality, the tumor recurrence and the decreased disease-free survival (Bauer et al., 1996; Fonseca et al., 2004; Quinn et al., 2005; Goto et al., 2008; Concato et al., 2009; Kluth et al., 2014).

The expression of Bcl-2 is often low in prostatic adenocarcinomas; it varied from 3.5% to 8.4% (Fonseca et al., 2004; Huges et al., 2005; Rubio et al., 2005). In the present study, this expression was 32% and was correlated with the death occurrence. The prognostic value of Bcl-2 has been demonstrated by some previous studies (Bauer et al., 1996; Concato et al., 2009). Concato et al. reported only 6% of tumors expressing Bcl-2 protein (Concato et al., 2009). This expression was associated to prostate cancer death (Concato et al., 2009). The expression of Bcl-2 was also associated with risk of disease recurrence (Bauer et al., 1996). and decrease of 5-years survival (Bachmann et al., 2011). The proliferation blocking and the apoptosis induction of prostate cancer cells are considered to be the therapeutic targets for prostatic cancer (Aghaei et al., 2011).

The prognostic value of the Ki-67 expression in prostate cancer has been demonstrated by several studies (Bantis et al., 2004; Inoue et al., 2005; Berney et al., 2009; Verhoeven et al., 2013). Inoue et al. demonstrated that Ki-67 is an independent prognostic factor associated with treatment failure and can predict tumor progression (Inoue et al., 2005). According to Verhoeven et al., the Ki-67 is a metastasis and death-occurred factor (Verhoeven et al., 2013). Berney et al. considered that the Ki-67 is a powerful biomarker for localized prostate cancer and directs the therapeutic strategies into radical or conservative treatment (Berney et al., 2009). As it is already widely used in routine pathology, these authors considered the Ki-67 as the most promising biomarker to be applied in routine practice (Berney et al., 2009).

Among the CDK inhibitors, only p27Kip1 was associated with the death occurrence in our study. Cohort studies have shown that the low expression of p27Kip1 may be an independent adverse prognostic factor of localized prostate cancer (Kuczyk et al., 1998; Freedland et al., 2003). In a multi-varied analysis of patients treated with radical prostatectomy, low p27Kip1 expression was an independent predictor of tumor recurrence (Ribal et al., 2003). Previously, Yang et al. showed that p27Kip1 is an independent predictor of disease-free survival, just after pathological stage, age, preoperative PSA level and Gleason score (Yang et al., 1998). However, a recent study showed that the loss of p27Kip1 expression had no effect on patient prognosis and prostate cancer progression (Sirma et al., 2013).

The PSA immunolabeling is prostate-specific and is a useful way to identify the primary prostatic origin of a metastasis and to differentiate invasive urothelial carcinomas from high-grade prostate carcinomas. PSA expression levels are often high reaching more than 90% of cancers cases in some studies (Zhigang et al., 2004; Chuang et al., 2007). In our study, 78% of prostate cancers expressed PSA. The level of PSA expression depends neither on age nor on serum PSA levels. Recently, studies suggested that the high PSA expression is associated with the early stages of prostatic cancer; however, the low PSA expression correlates with the metastatic progression of this cancer (Larkin et al., 2012).

In contrast to the breast cancer, the HER2 expression significance and prognostic value are controversial in prostate cancer. In most studies, HER2 expression was low. Only 12% of tumor cases expressed HER2 in our study. As observed here, no correlation was reported with serum PSA level, tumor size, clinical stage, Gleason score and tumor recurrence (Calvo et al., 2003; Fonseca et al., 2004; Zhang et al., 2011; Zahir et al., 2014). However, Isharwal et al. considered those patients expressing HER2 and having a high-DNA index are at high-risk of disease progression, metastasis and death from prostate

cancer (Isharwal et al., 2008). In addition, these authors suggested the use of HER2 expression and DNA index with clinicopathological parameters for predicting the long-term prognosis of prostate cancer (Isharwal et al., 2008).

In the current study, 90% of tumor cases expressed E-Cadherin and no correlation was observed with age, serum PSA, the degree of tumor differentiation and the death occurrence. However, Umbas et al. found a significant correlation between the low expression of E-Cadherin and the histological grade, the tumor stage, the presence of distant metastases and the overall 5-years survival in patients with localized prostate adenocarcinoma treated by radical prostatectomy (Umbas et al., 1993). More recently, Lazari et al. observed a low or even an absent E-Cadherin expression associated with a poor prognosis and increased cancer mortality rates (Lazari et al., 2013).

In conclusion, our study confirmed previous findings regarding the prognostic interest of clinico-pathological features mainly the advanced age, the Gleason score, the degree of tumor differentiation, the perineural invasion and the metastasis occurrence. In addition, the expression of p53, Bcl-2, Ki-67 and p27Kip1 might help in the management of prostate cancer. Thus, p53, Bcl-2, Ki-67 and p27Kip1 could be useful additional prognostic markers for prostate cancer. The use of these proteins in clinical practice can improve prognosis prediction, disease screening and treatment response of prostatic cancer.

Acknowledgements

References

Aghaei M, Panjehpour M, Karami-Tehrani F, et al (2011). Molecular mechanisms of A3 adenosine receptor-induced G1 cell cycle arrest and apoptosis in androgen-dependent and independent prostate cancer cell lines: involvement of intrinsic pathway. *J Cancer Res Clin Oncol*, **137**, 1511-23.

Baade PD, Yu XQ, Smith DP, et al (2015). Geographic disparities in prostate cancer outcomes--review of international patterns. *Asian Pac J Cancer Prev*, **16**, 1259-75.

Bachmann HS, Heukamp LC, Schmitz KJ, et al (2011). Regulatory BCL2 promoter polymorphism (-938C>A). is associated with adverse outcome in patients with prostate carcinoma. *Int J Cancer*, **129**, 2390-9.

Bantis A, Giannopoulos A, Gonidi M, et al (2004). Expression of p120, Ki-67 and PCNA as proliferation biomarkers in imprint smears of prostate carcinoma and their prognostic value. *Cytopathology*, **15**, 25-31.

Bashir MN (2015). Epidemiology of Prostate Cancer. *Asian Pac J Cancer Prev*, **16**, 5137-41.

Bauer JJ, Sesterhenn IA, Mostofi FK, et al (1996). Elevated levels of apoptosis regulator proteins p53 and bcl-2 are independent prognostic biomarkers in surgically treated clinically localized prostate cancer. *J Urol*, **156**, 1511-6.

Bensalah K, Lotan Y, Karam JA et al (2008). New circulating biomarkers for prostate cancer. Prostate Cancer Prostatic

Dis, 11, 112-20.

Berney DM, Gopalan A, Kudahetti S, et al (2009). Ki-67 and outcome in clinically localised prostate cancer: analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. *Br J Cancer*, **100**, 888-93.

Boström PJ, Bjartell AS, Catto JW, et al (2015). Genomic predictors of outcome in prostate cancer. *Eur Urol*, **68**, 1033-44.

Calvo BF, Levine AM, Marcos M, et al (2003). Human epidermal receptor-2 expression in prostate cancer. *Clin Cancer Res*, **9**, 1087-97.

Chuang AY, DeMarzo AM, Veltri RW, et al (2007). Immunohistochemical differentiation of high-grade prostate carcinoma from urothelial carcinoma. *Am J Surg Pathol*, **31**, 1246-55.

Concato J, Jain D, Uchio E, et al (2009). Molecular markers and death from prostate cancer. *Ann Intern Med*, **150**, 595-603.

Daniyal M, Siddiqui ZA, Akram M, et al (2014). Epidemiology, etiology, diagnosis and treatment of prostate cancer. *Asian Pac J Cancer Prev*, **15**, 9575-8.

Esfahani M, Ataei N, Panjehpour M (2015). Biomarkers for Evaluation of Prostate Cancer Prognosis. *Asian Pac J Cancer Prev*, **16**, 2601-11.

Fonseca GN, Srougi M, Leite KR, et al (2004). The role of HER2/neu, BCL2, p53 genes and proliferating cell nuclear protein as molecular prognostic parameters in localized prostate carcinoma. *Sao Paulo Med J*, **122**, 124-7.

Freedland SJ, deGregorio F, Sacoolidge JC, et al (2003). Preoperative p27 status is an independent predictor of prostate specific antigen failure following radical prostatectomy. *J Urol*, **169**, 1325-30.

Globocan (2012). Assessed the 23rd April 2016, http://globocan.iarc.fr/Default.aspx.

Goto T, Nguyen BP, Nakano M, et al (2008). Utility of Bcl-2, P53, Ki-67, and caveolin-1 immunostaining in the prediction of biochemical failure after radical prostatectomy in a Japanese population. *Urol*, **72**, 167-71.

Ho CC, Seong PK, Zainuddin ZM, et al (2013). Retrospective study of predictors of bone metastasis in prostate cancer cases. *Asian Pac J Cancer Prev*, **14**, 3289-92.

Hughes C, Murphy A, Martin C, et al (2005). Molecular pathology of prostate cancer. *J Clin Pathol*, **58**, 673-84.

Inoue T, Segawa T, Shiraishi T, et al (2005). Androgen receptor, Ki67, and p53 expression in radical prostatectomy specimens predict treatment failure in Japanese population. *Urol*, **66**, 332-7.

Isharwal S, Miller MC, Epstein JI, et al (2008). Prognostic value of Her-2/neu and DNA index for progression, metastasis and prostate cancer-specific death in men with long-term follow-up after radical prostatectomy. *Int J Cancer*, **123**, 2636-43.

Kluth M, Harasimowicz S, Burkhardt L, et al (2014). Clinical significance of different types of p53 gene alteration in surgically treated prostate cancer. *Int J Cancer*, **135**, 1369-80.

Kuczyk MA, Serth J, Bokemeyer C, et al (1998). The prognostic value of p53 for long-term and recurrence-free survival following radical prostatectomy. *Eur J Cancer*, **34**, 679-86.

Larkin SE, Holmes S, Cree IA, et al (2012). Identification of markers of prostate cancer progression using candidate gene expression. *Br J Cancer*, **106**, 157-65.

Lazari P, Poulias H, Gakiopoulou H, et al (2013). Differential immunohistochemical expression of CD44s, E-cadherin and β-catenin among hyperplastic and neoplastic lesions of the prostate gland. *Urol Int*, **90**, 109-16.

Missaoui N, Trabelsi A, Parkin DM, et al (2010). Trends in the incidence of cancer in the Sousse region, Tunisia, 1993-2006.

Nabiha Missaoui et al

Int J Cancer, **127**, 2669-77.

Maru N, Ohori M, Kattan MW, et al (2001). Prognostic significance of the diameter of perineural invasion in radical prostatectomy specimens. *Hum Pathol*, **32**, 828-33.

Molinié V, Beuzeboc P, Mahjoub WK et al (2008). Molecular biology and prostate cancer: evolution or revolution? *Ann Pathol*, **28**, 354-62.

Molinié V (2008). Gleason's score: update in 2008. *Ann Pathol*, **28**, 350-53.

Quinn DI, Henshall SM, Sutherland RL (2005). Molecular markers of prostate cancer outcome. *Eur J Cancer*, **41**, 858-87.

Ramírez ML, Nelson EC, Evans CP (2008). Beyond prostate-specific antigen: alternate serum markers. *Prostate Cancer*, **11**, 216-29.

Ribal MJ, Fernandez PL, Lopez-Guillermo A, et al (2003). Low p27 expression predicts biochemical relapse after radical prostatectomy in patients with clinically localised prostate cancer. *Anticancer Res*, **23**, 5101-6.

Salomon L, Azria D, Bastide C, et al (2010). Recommendations Onco-Urology 2010: Prostate cancer. *Prog Urol*, 20, 217-51.

Sirma H, Broemel M, Stumm L, et al (2013). Loss of CDKN1B/p27Kip1 expression is associated with ERG fusion-negative prostate cancer, but is unrelated to patient prognosis. *Oncol Lett*, **6**, 1245-52.

Rubio J, Ramos D, Lopez-Guerrero JA, et al (2005). Immunohistochemical expression of Ki-67 antigen, cox-2 and Bax/Bcl-2 in prostate cancer; prognostic value in biopsies and radical prostatectomy specimens. *Eur Urol*, **48**, 745-51.

Umbas R, Isaacs WB, Bringuier PP, et al (1994). Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res*, **54**, 3929-33.

Van Dong H, Lee AH, Nga NH, et al (2014). Epidemiology and prevention of prostate cancer in Vietnam. *Asian Pac J Cancer Prev*, **15**, 9747-51.

Verhoven B, Yan Y, Ritter M, et al (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. *Int J Radiat Oncol Biol Phys*, **86**, 317-23.

Wang K, Peng HL, Li LK (2012). Prognostic value of vascular endothelial growth factor expression in patients with prostate cancer: a systematic review with meta-analysis. *Asian Pac J Cancer Prev*, **13**, 5665-9.

Wolff AC, Hammond ME, Hicks DG, et al (2013). Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*, **31**, 3997-4013.

Yamashita K, Denno K, Ueda T, et al (1993). Prognostic significance of bone metastases in patients with metastatic prostate cancer. *Cancer*, **71**, 1297-302.

Yang RM, Naitoh J, Murphy M, et al (1998). Low p27 expression predicts poor disease-free survival in patients with prostate cancer. *J Urol*, **159**, 941-5.

Zahir ST, Tafti HF, Rahmani K (2014). Overexpression of HER-2/neu in patients with prostatic adenocarcinoma. *Asian Pac J Cancer Prev*, **15**, 6425-8.

Zhang YF, Guan YB, Yang B, et al (2011). Prognostic value of Her-2/neu and clinicopathologic factors for evaluating progression and disease-specific death in Chinese men with prostate cancer. *Chin Med J (Engl)*, **124**, 4345-9.

Zhigang Z, Wenlv S (2004). Prostate stem cell antigen (PSCA). expression in human prostate cancer tissues and its potential role in prostate carcinogenesis and progression of prostate cancer. *World J Surg Oncol*, **2**, 13.

100.0

6.3

75.0

56.3

50.0

25.0

31.3

0

Newly diagnosed without treatment