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Original article

Survival predictors in patients with prostate adenocarcinoma with hormonal blockade

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ABSTRACT

Ki-67 index and clinical-pathological factors such as the Gleason score and the presence of neuroendocrine differentiation have been used for predicting survival in patients with prostate cancer. We examined prostate tissue from 45 patients with advanced prostate cancer who were treated with maximal androgen blockade and analysed their cancer-specific survival (CSS). We assessed the Gleason index, performed an immunohistochemical analysis of Ki-67 (MIB-1) and determined the presence of neuroendocrine differentiation (chromogranin A). A survival study was conducted using Kaplan-Meier curves (log-rank test) and a Cox regression analysis. Twenty-four patients (53.3%) died from the disease, with a mean follow-up of 68.7 ± 7.7 months (56.6% CSS at 5 years and 31.8% at 10 years). In the univariate analysis, survival was associated with an interquartile distribution of Ki-67 (0–5, 6–12%, 13–25%, >25%; log-rank, p = 0.01), Gleason 5 (total index 9–10; log-rank, p = 0.002) and the presence of metastases during the diagnosis (M1; log-rank, p = 0.004) but not to cT category (T3–T4; log-rank, p = 0.26) or neuroendocrine differentiation (immunohistochemically positive tumour cell nests; log-rank, p = 0.46). The multivariate analysis revealed that a Ki-67 index ≤12% (HR, 0.22; p = 0.0009) and the absence of metastases (M0) during diagnosis (HR, 0.17; p = 0.0002) were protective factors in this population. In conclusion, Ki-67 proliferation index and the lack of metastases at diagnosis predict CSS in patients with advanced prostate cancer who undergo hormonal blockade. Neuroendocrine differentiation in tumour tissue had no prognostic value in this study.

1. Introduction

Prostate cancer is the most common malignancy in men in the developed world and the third leading cause of death among these men [1]. The clinical outcome of this malignancy is unfavourable when there is a conversion of a hormonal sensitivity condition to another of castration resistance [2]. A better understanding of the molecular changes involved in this outcome is essential to predicting the outcome and planning a therapeutic strategy for these patients. The silencing of specific genes through promoter hypermethylation is under investigation as a new marker of advanced prostate cancer progression [3] and specifically for patients treated with hormonal blockade [4]. Defining failure of conventional androgenic deprivation (AD) therapy ahead of time can be a key step, especially these days with the advent of new hormonal therapies and emerging treatments for castration-resistant prostate cancer (CRPC) [5].

It has been suggested that early detection of neuroendocrine activity in prostate cancer could anticipate the diagnosis of hormone refractoriness and even justify changes in the therapeutic approach for patients after surgery [6]. Neuroendocrine activity appears to be involved in the progression of a dependence condition to hormonal independence in prostate cancer. The continuous use of androgenic ablation therapy can cause neuroendocrine system hyperactivation in the prostate tissue. The products of this system can act as factors that inhibit the neoplastic cell apoptosis, inducing androgenic independence and disease progression

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[7]. However, the value of neuroendocrine differentiation (NED) in predicting the prognosis of prostate cancer, especially in patients undergoing antiandrogen therapy, is a controversial subject [8,9].

The Ki-67 index, determined by the expression of the nuclear protein complex MIB-1 in the various cell cycle phases of proliferate cells (G1, S and G2-M) but not at rest (G0), is a marker regularly used to determine and quantify cell proliferation in various tumours [10,11]. With respect to prostate cancer, Ki-67 has been employed to differentiate benign from malignant prostate tissue and to assess the prognosis and response to treatment [6,12].

Sustained hormonal blockade is still the first-line treatment for delaying the progression of metastatic or locally advanced disease. In this study, we assess the role of conventional histopathology markers such as the Gleason score, the presence of NED and the cell proliferation index when predicting survival for patients who undergo AD therapy.

2. Materials and methods

The authors declare that all the experiments carried out in this study comply with current Spanish and European Union legal regulations.

A retrospective analysis of a sample of 45 patients with advanced prostate cancer who underwent total androgen blockade therapy (luteinizing hormone-releasing hormone analogue or orchiectomy combined with an oral antiandrogen). The tissue for histopathological analysis was obtained from specimens from radical prostatectomy (n = 24) or transurethral resection of the prostate (TURP) (n = 21). For all patients in whom the disease presented as metastatic at diagnosis, the material came from TURP.

The patients' follow-up was conducted every 3–6 months, mainly to determine prostate-specific antigen (PSA) levels and conduct imaging studies. Total androgen blockade therapy was started immediately when surgery was evidently not curative (lymphocytic involvement and/or incomplete resection) (n = 27). The therapy was deferred (n = 18) if biochemical progression was confirmed, always with a progressive increase in PSA levels and a doubling time of <3 months. The primary study objective was the patients' cancer-specific survival (CSS). The survival time under analysis was measured from the start of total androgen blockade therapy in all cases (not from the moment surgical specimens were obtained) to the patient's death due to the disease.

The study of the samples was performed by a uropathologist. Formalin-fixed paraffin-embedded tissues were processed for immunohistochemical analysis following routine methods. Immunostainings with Ki67 (monoclonal MIB-1, dilution: 1/50; DAKO, Glostrup, Denmark) and chromogranin A (monoclonal DAK-A3, dilution: 1/100; DAKO, Glostrup, Denmark) were performed in automated immunostainers (EnVision FLEX, Dako Autostainer Plus; Dako, Glostrup, Denmark). Tris-EDTA was used for antigen retrieval. Negative controls were slides not exposed to the primary antibody, and these were incubated in PBS and then processed under the same conditions as the test slides.

The positive nuclear staining for MIB-1 was evaluated in percentages, thereby defining the Ki-67 proliferation index for each case as the proportion of stained nuclei compared with the total number of cellular nuclei. Interquartile ranges were established by sections (0–5%, 6–12%, 13–25%, >25%). NED was assessed using a semiquantitative approach described by di Sant'Agnese and Mesy Jensen [13]. Each tumour was classified as negative NED when immunostaining of the assessed marker was not detected or when the cells with positive staining were dispersed without forming groups. The tumour was classified as positive NED when there was at least one grouping of chromogranin A-positive cells.

The patients were followed-up to death due to cancer or censored for still being alive or having died from some other cause. The CSS analysis was performed using the Kaplan-Meier method, evaluating the significance with the log-rank test. The Cox regression analysis was performed using a stepwise variable selection model, with an entry threshold of p = 0.15 and an enter/remove criterion of p = 0.20. The statistical analysis was conducted using SAS 9.3 software (2002–2010, SAS Institute Inc., Cary, NY, USA).

3. Results

The patients had a mean age at diagnosis of 68.7 ± 7.7 years (range 57–85) and a PSA level of 77.9 ± 155.9 ng/mL (range 0.3–820.2). The mean time between obtaining the surgical specimen and the start of hormone therapy was 3.7 ± 11.9 months (range 1–21). A total of 24 patients (53.3%) died due to the disease within a mean period of 49 months (95% CI 38.3–59.7) after the start of total androgen blockade therapy. The CSS at 1, 5 and 10 years was 95.5% (95% CI 83.4–98.9), 50.5% (95% CI 33.9–64.9) and 31.8% (95% CI 13.7–51.7), respectively (Fig. 1A).

The disease was interpreted as clinically localised in only 9 cases (20%), while the rest were clinically advanced at diagnosis. Metastases were detected at diagnosis in 11 cases (24.4%). The CSS of M0 patients was 60.7% (95% CI 41–75.7) at 60 months, while the CSS for M1 patients was 15.3% (95% CI 1–46.4), a difference that was statistically significant (log-rank; p = 0.004) (Fig. 1B). For 19 patients of this series (43.2%), at least one of the Gleason patterns (primary or secondary) was 5 (Gleason 9–10). The CSS for patients with Gleason scores ≤8 was 68.6% (95% CI 45–83.7) at 60 months vs. 27% (95% CI 9.1–49) for those with Gleason scores of 9–10. This difference was also statistically significant (log-rank; p = 0.002) (Fig. 1C).

Positive IHC staining for chromogranin A in the tissue tumour was detected in 16 cases (35.6%). For these patients, the CSS was 41.7% (95% CI 17.4–64.6) at 60 months, while for those cases in which this differentiation was not detected, the CSS was 55.7% (33.9–72.9) during the same period. The difference in this case was not statistically significant (log-rank; p = 0.46) (Fig. 1D). In one of the cases, the MIB-1 immunostaining could not be assessed. In the other cases, the Ki-67 index had the following distribution: 0–5% in 22 cases (50%), 6–12% in 4 (9.1%), 13–25% in 10 (22.7%) and >25% in 8 (18.2%) (Fig. 1E). There was an ordinal association between the Ki-67 and Gleason indices (Cochran-Armitage; p = 0.06). There was no association between NED and Ki-67 (Fisher; p = 1.0). The CSS for patients with a Ki-67 index ≤12% was 63% (95% CI 41.7–78.4) at 60 months, while for those with an index >12%, the CSS was 16.7% (95% CI 2.7–41.3) during the same period. This difference was statistically significant (log-rank; p = 0.001) (Fig. 1F).

Table 1 shows the univariate and multivariate analysis of the clinical-pathological variables that predict CSS in the patients with prostate cancer in hormonal blockade. NED does not appear to affect the risk of progression and death for patients with AD. The cT category of the primary tumour at the time of diagnosis also had no impact on the risk. In the regression model, the variables with an independent predictive character were Ki-67 and metastases at diagnosis. Thus, in this series, the cases with a Ki-67 proliferation index ≤12% had a lower risk of death (HR, 0.22; 95% CI 0.09–0.54; p = 0.0009). Similarly, the patients who started hormonal blockade without presenting metastases at the time of diagnosis also had better prognoses (HR, 0.17; 95% CI 0.07–0.42; p = 0.0002).

4. Discussion

CRPC is a clinical condition for which new treatment options are emerging. It is essential to understand the roots underlying the development of the castration resistance phenomenon, the clinical