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PROSTATE CANCER: HIGH KI-67 DOES NOT PREDICT HORMONE RESISTANCE

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ABSTRACT

Objectives: To evaluateKi-67 expression in metastatic prostate tumors and correlate it with overall survival and progression-free survival data in patients submitted to central hormone blockade. **Methods:** Retrospective analysis of medical records and review of biological material of 45 patients with metastatic prostate cancer who were diagnosed and followed up at the São Paulo Medical School, Universidade Federal de São Paulo, São Paulo, Brazil, from 2000 to 2013. **Results:** Bone metastasis was present in 97% of the patients, and visceral metastases more frequently involved the lungs (42.8%) and bone marrow (28.6%). A high-volume disease involving four or more bone lesions, with at least one being extra-axial and/or visceral, was found in 66% of the patients. All participants who began cancer treatment were submitted to central hormone blockade, and the mean time for reaching prostate-specific antigen nadir was 13.6 months. Progression-free survival of 23.8 months and overall survival of 42.5 months were observed. The maximum Ki-67 median for the high tumor burden group was 6.67, and it was 8.24 (p=0.50) for the group with low disease volume. Evaluation of the relationship between maximum Ki-67 and progression-free survival showed a relative risk of 1.03 (95% CI: 0.979-1.094, p=0.23). No correlation was found between maximum Ki-67 and overall survival, with a relative risk of 1.03 (95% CI: 0.935-1.140). **Conclusions:** Cell division rate analysis, the simplest measure of change in cell growth associated with neoplastic transformation, did not correlate with prognosis of metastatic prostate cancer.

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INTRODUCTION

Ki-67 protein was described for the first time in 1980. It is a nuclear antigen expressed in proliferation cells, has a half-life of 1 to 1.5 hours, is present during the whole cell cycle (phases G1, S, G2, and M), reaching maximum levels in the G2 and M phases, and is not expressed in quiescent cells (G0). Therefore, it is used as a marker of neoplastic tissue growth fraction, quantified by using immunohistochemistry methods, and described as a percentage of cells that show activity in a tissue sample (1). Several clinical-pathological parameters have been investigated in prostate cancer and nomograms have been designed to better stratify and predict disease progression risk, especially for localized neoplasia. In this case, staining with Ki-67 has consistently shown prognostic value, and has been tested in men treated with radiation and surgery, as well as those managed conservatively, without a definitive therapy (2), (3), (4), (5). Evaluation of Ki-67 expression was different according to risk groups in the National Comprehensive Cancer Network for localized prostatic neoplasia. The means obtained for low, intermediate, and high-risk groups were 5.1%, 7.4%, and 12.0%, respectively (2). Additionally, it was possible to state that the Ki-67 value was an independent predictor of disease-specific mortality, distant metastasis, and biochemical failure in patients with low or intermediate-risk localized prostate cancer (2),(3),(4),(5),(6). The variations in this protein according to the T stage and prostate-specific antigen (PSA) values were not significant (2). The average intraprostatic Ki-67 variation (defined as the highest Ki-67 value minus the lowest Ki-67 value in two or more positive samples in the prostate biopsy) was 2.6%, 5.3%, and 10.9% for low, intermediate, and high-risk groups, respectively. The intralesional Ki-67 variation also showed a tendency toward heterogeneity in high-risk patients. In this group, the variation found was 8.1% whereas the variation in Ki-67 values in low-risk patients was 1.1%. Prostatic lesions considered dominant and nondominant showed coincident Ki-67 values in 30% of the cases. The presence of the highest Ki-67 indexes in nondominant lesions in up to 40% of the studied cases was described, which suggested that biopsy of index lesions in isolation may not have reliable representativeness for Ki-67 values (2). Different cutoff levels for Ki-67 expression have been defined in studies that examined patients with localized prostate cancer after radiation therapy, associated with hormone therapy or not (3), (4), (5), (6), (7). In the study conducted by the Radiation Therapy Oncology Group, a Ki-67 value higher than 6.2% was an independent predictor of

disease-specific mortality (HR: 2.91), distant metastasis (HR: 3.62), and biochemical failure (HR: 3.29) in low or intermediate-risk localized prostate cancer patients (3). Another study involving intermediate and high-risk patients demonstrated that Ki-67 values equal to or higher than 7.1% were related to adverse clinical outcomes (4). A third study indicated that Ki-67 indexes higher than 3.5% were strongly predictive of cause-specific mortality and distant metastasis in high-risk patients, as shown by multivariate analysis (5). The Ki-67 indexes measured for bone metastases were comparable to those reported in several studies that evaluated primary prostate tumors, and a marked overlap was observed for groups with high and low-volume disease, as well as for different Gleason differentiation groups, which was possibly related to the small sample size(6). Based on the medical literature, it is important to stress that there is no description of a difference between average Ki-67 indexes in material collected during transurethral resection of the prostate and that obtained by means of needle biopsy (7).

The results above show that immunohistochemical detection of Ki-67 has consistently been shown to have the potential for the use of this protein as an independent marker in patients with localized prostate cancer. At present, there is no prospective validation of cutoffs for this scale. Data restricted to patients with metastatic prostate cancer, collected when diagnosis was confirmed, were not found in recent studies. The current trend in the treatment of metastatic prostate cancer is predominantly based on the existing tumor volume, with benefits in overall survival when chemotherapy is applied in high-volume disease (8),(9). In this scenario, tumor heterogeneity becomes important information when facing the possibilities for treating this patient. An ideal marker must be measurable at the time of diagnosis, show high sensitivity and specificity in distinguishing between indolent and lethal prostate cancer and be measured with reproducibility by different experimenters and in different institutions. Consequently, the attempt to evaluate a known immunohistochemical parameter such as the Ki-67 protein is justified, with the view that higher growth rates would indicate a worse prognosis. Based on existing research, it is possible to conclude that integrating biomarkers, including the Ki-67 protein, into the existing risk stratification system and correlating them with clinical outcomes will help customize current treatment options. We aim to evaluate Ki-67 expression in tumors of patients with metastatic prostate cancer submitted to central hormone blockade and correlate it with overall (OS) and progression-free survival (PFS).

MATERIALS AND METHODS

The authors collected retrospective clinical data from medical records and reviewed microscope slides of patients with metastatic prostate cancer diagnosed and treated at São Paulo Hospital /Federal University of São Paulo (SPH/UNIFESP) from 2000 to 2013, which totaled 45 patients. Initially, 12 patients were excluded from this sample, because of localized cancer that had not been treated, had received their treatment at another institution or did not have a confirmed diagnosis of prostate neoplasia. Subsequently, four patients were excluded for not having their biological material at the institution and three samples were excluded because they did not contain enough material for proper histochemical evaluation and yet another patient was discarded for not having undergone treatment for metastatic disease. Consequently, it was possible to correlate Ki-67 expression and epidemiological data of 25 cases. The present cross-sectional study evaluated material originating from prostate needle biopsy, transurethral resection of the prostate, prostatectomy, and the involved metastasis sites. All the analysis were performed at the Department of Pathology of SPH/UNIFESP over the same period. Immunohistochemical reactions were carried out in 3-micrometer histological sections obtained from samples fixed in formaldehyde, embedded in paraffin, and laid on slides prepared with saline solution. Primary antibody for Ki-67 (ready-to-use MIB-1 clone, Dako) was used. Reactions were obtained with a DakoImmunostainer Link 48 automatized immunostainer, on Flex mode, with low pH. The chosen positive control (palatine tonsil) worked properly.

The slides were evaluated by a pathologist whose area of activity is uropathology and who had no knowledge about the clinical data of the studied patients. Diagnosis and histological grade were reviewed in all the cases. Histological grade was not applied to metastatic lesions. For each case, two cell proliferation indexes were obtained by applying a common proportional count method (ratio of the number of stained neoplastic cells and the total number of neoplastic cells) in one high-power field. The maximum index was obtained in the highest proliferation spot (hot spot), and the minimum value was measured in the lowest proliferation spot (cold spot) (Figure 1). Regarding the samples of selected patients, the mean age of the patients at diagnosis, presence of symptoms, biopsy site and volume of disease were evaluated. The correlation between the minimum and maximum Ki-67 values sampled was assessed by Spearman's test. The relationship between maximal Ki-67 and PFS was determined by Cox regression. In the same way, it was possible to correlate the maximal Ki-67 value and OS. To test whether there was a correlation between Ki-67 and PSA at diagnosis, we performed linear regression.

RESULTS

The median age at diagnosis was 65 years-old, 96% of the patients were symptomatic and one-third needed to use opioids. The prostate primary site was the prevalent assessed site (88.9%), and over 50% of the patients were submitted to needle biopsy. In the analyzed sample, 34.6% of the patients had locally advanced disease and 42.3% presented lymph node metastasis. Bone metastases were present in 97% of the patients, and visceral metastases more frequently involved the lungs (42.8%) and bone marrow (28.6%). High-volume disease, involving four or more bone lesions, with at least one being extra-axial and/or visceral, was observed in 66% of the patients. The median value of maximum Ki-67 in the group with high tumor load was 6.67%, whereas patients with low-volume disease had a value of 8.24% (p=0.50).

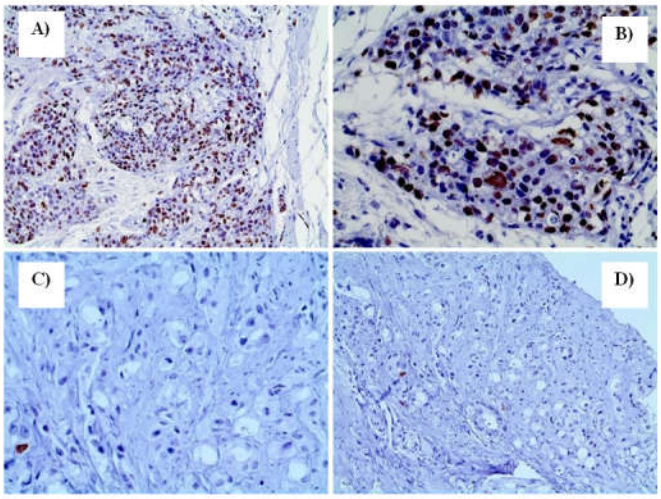

Figure 1. Expression of Ki-67 in different samples: (A) High Ki-67: Immunoperoxidase 200x. (B) High Ki-67: 400x immunoperoxidase. (C) Low Ki-67: Immunoperoxidase 200x. (D) Low Ki-67: 400x immunoperoxidase

All the participants were submitted to central hormone blockade as the initial therapy, and the average time to reach the prostate-specific antigen nadir was 13.6 months and median PFS was 23.8 months. During disease progression, which occurred in 25 patients, the main progression sites were bones and lymph nodes. After development of hormone resistance, five patients discontinued treatment. Among those who received treatment, 85% were started on full hormone blockade and 15% received chemotherapy. Overtime, 42.4% of the patients received chemotherapy at some point. The OS of this population was 42.5 months. The average Ki-67 in cold spots was 2.17% (0.36% – 9.4%), whereas the average index obtained in hot spots was 10.92% (1.04% – 58.2%).

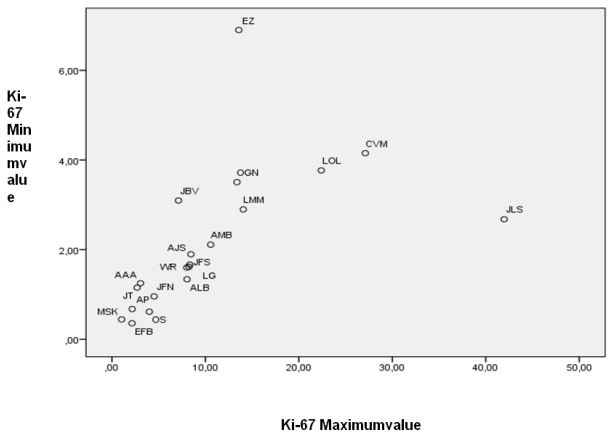

Figure 2. Correlation betweenKi-67 minimum and maximum values sampled from the evaluated patients

The mean Ki-67 intra-sample variability was 8.33%. The correlation found between minimum and maximum Ki-67 values sampled in Spearman's test was 0.883 (p<0.0001), which is considered strong (Figure 2). The relationship between maximum Ki-67 value and PFS, by applying Cox regression analysis, showed a relative risk (RR) of 1.03 (95% CI: 0.979 – 1.094, p=0.23). No correlation between maximum Ki-67 and OS was found, RR value was 1.03 (95% CI: 0.935 – 1.140) (Table 1).

Table 1. Correlation between sampled Ki-67 maximum values and overall (OS) or progression-free survival (PFS) in the evaluated patients

Variable	N	RR (95% CI)	p value
Ki-67 vs. PFS	27	1.0349 (0.9787 – 1.0942)	.228
Volume vs. PFS	24	0.1668 (0.0206 – 1.3538)	.093
Ki-67 vs. OS	28	1.0329 (0.9354 – 1.1406)	.5219

Linear regression analysis was carried out to test whether there was a correlation between Ki-67 and PSA values when diagnosis was made. No correlation was observed, with r=0.1034 (p=0.615). Additionally, it was not possible to correlate Ki-67 maximum values with Gleason classification (G7 with Ki-67=6.1, G8 with Ki-67=7.47, and G9 with Ki-67=10.81; p=0.412).

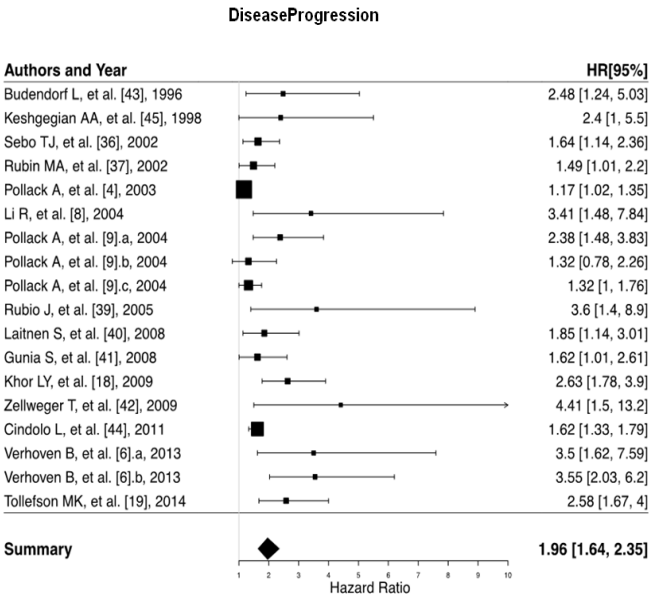

Figure 3. Correlation between sampled Ki-67 values and disease progression in several clinical trials involving patients with localized prostate cancer

DISCUSSION

Analyzing cell division rate by Ki-67, the simplest measure of alteration in cell growth associated with neoplastic transformation, does not reflect the behavior and prognosis of metastatic prostate cancer. Patients with high tumor load showed a median of 6.67% for maximum Ki-67, and the value for patients with low-volume disease was 8.24%, with no difference between the groups, in contrast with the expected result. An aspect that can be questioned, however, is the time of evolution of the cancer status. It is known that, in the period between 2000 and 2013, patients were belatedly referred to specialized medical services in Brazil. It was only in 2014 that the Brazilian Ministry of Health issued a decree to establish a maximum deadline of 60 days for cancer treatment to be initiated in the Brazilian Unified Health System (10). This delay in beginning cancer treatment may have contributed to the low level found for maximum Ki-67 in the high-volume disease group, which directly affected OS and PFS results. Raymond et al. (11) reported an average Ki-67 growth fraction of 16.3%, varying from 6% to 53%, for prostate cancer in several stages. According to Gallee et al. (12), poorly differentiated prostate adenocarcinomas showed a mean value of 2.5% (0.6%-7.1%), and well-differentiated had a mean value of 2.9% (0.7%-4.0%). Regarding other human epithelial tumors, the highest Ki-67 indexes described so far are those for lung cancer (small-cell carcinoma, with 65%, and adenocarcinoma, with 24.6%), breast cancer (16.7%), cervical cancer (30.7%), and high-degree colon adenocarcinoma (43.8%)(1).

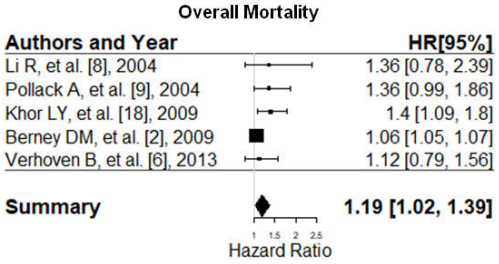

Figure 4. Correlation between sampled Ki-67 values and overall mortality in several clinical trials involving patients with localized prostate cancer

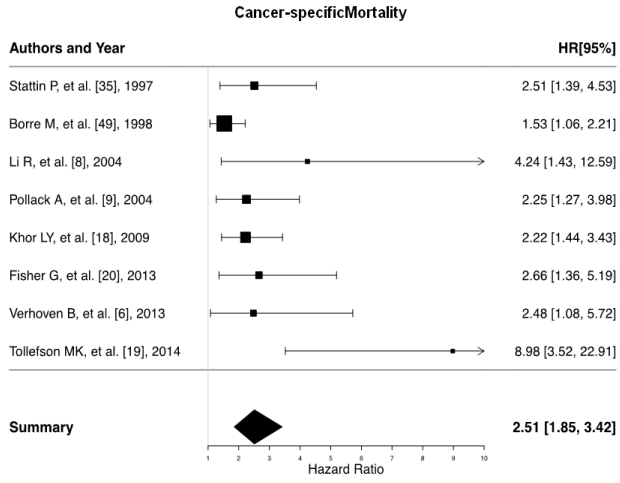

Figure 5. Correlation between sampled Ki-67 values and cancer-specific mortality in several clinical trials involving patients with localized prostate cancer

The reasons that Ki-67 indexes are lower in prostate cancer cases, as illustrated by the present study, are not clear. It is possible that the high indexes found by Raymond et at. (11) can be explained by the method used, which considered both nuclear and cytoplasmic Ki-67, in opposition to the technique adopted in the present and most other studies, which only measures the expression of nuclear Ki-67. It is

not possible to rule out the hypothesis that the lack of correlation in the present study was caused by the reduced sample size and by bias in retrospective data collection. It is necessary to question whether the evaluation of dominant lesions in the primary site really shows the highest Ki-67 values when prostate tissue is obtained from metastatic patients. In addition, it is possible to consider the occurrence of different results if the biopsy had been performed at secondary lesions and, by definition, neoplastic populations with more aggressive potential and higher chances to metastasize. At present, the literature shows only one study that examined nontreated patients with metastatic prostate cancer reporting Ki-67 indexes for bone metastases that were comparable to those obtained in many studies on prostate tumors (6) and, consequently, it is not possible to state that these values are equivalent. In the present study, those three cases in which the anatomopathological material was extracted from secondary bone lesions, the Ki-67 indexes were 3.18%, 10.56%, and 22.39% (average value of 12.04%), whereas the values measured in material originating from biopsies or transurethral resection of the prostate ranged from 1.03% to 58.22% (average value of 10.10%).

These differences in the ways to standardize and measure Ki-67 values, and the choice of evaluation site, could help explain the discrepancies in results found in the present study regarding localized prostate cancer, for which immunohistochemical Ki-67 detection has consistently shown potential as an independent marker of disease-specific mortality, distant metastasis, and biochemical failure. Figure 3-5 summarizes the results of several studies and shows the correlations between Ki-67 indexes and disease progression, with an RR of 1.96 (1.64-2.35), an overall mortality of 1.19 (1.02-1.39), and a cancer-specific mortality of 2.51 (1.85-3.42) in the localized prostate cancer scenario, respectively. Data collected in the present study corroborated the results of a 1991 study carried out by Sadi et al. (13) with 17 patients diagnosed with metastatic prostate cancer and submitted to biopsy of the primary tumor right before the beginning of central hormone blockade. Interestingly, this study did not show a correlation between Ki-67 indexes and expression of androgen receptors in tumor cells. The Ki-67 values were 3.5% and 3.1%, respectively, for tumors classified as weak androgen receptor expressers and those categorized as strong androgen receptor expressers. The patients were analyzed into two categories, considering: poor responders (average PFS of 9 months) and good responders (average PFS of 36 months), and comparison of growth fractions in these patient groups indicated no difference. Good responders showed a mean Ki-67 index of 3.1% and poor responders had a mean Ki-67 index of 3.5% (13).

Combined analysis with this investigation (13) and the present study did not show statistical significance for the correlation between Ki-67 values and PFS (p=0.42), which reinforced the validity of the findings obtained by each group of authors. It is likely that metastatic prostate cancer is so complex that no single parameter can provide enough information on the behavior of the tumor and predict the individual's clinical response to therapy. Contrary to what happens in patients with localized prostate cancer under follow-up after treatment, it is possible that tumor load is an important factor in patients with metastatic prostate cancer, affecting time until progression. Additionally, although Ki-67 expression evaluates the growth fraction, the actual duplication time of the tumor depends on the cell death rate. If the cell proliferation rate were balanced by a high cell death rate, promoted, for instance, by a good response to hormonal castration, Ki-67 data in isolation would overestimate the tumor growth rate (13), (14).

CONCLUSION

The results of the present study allow for the conclusion that integrating the Ki-67 biomarker into the existing risk stratification system designed for patients with metastatic prostate cancer and correlating Ki-67 values with clinical outcomes were not possible.

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