

Prognostic value of Ki-67 in patients with hypertension and prostate cancer: A real-world study in a Chinese population

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Abstract

Background

Prostate cancer is the second most common malignancy in males worldwide, with high mortality, especially when combined with hypertension. Ki-67 is one of the most reliable markers of growth for neoplastic human cell populations. However, the prognostic value of Ki-67 in patients with hypertension and prostate cancer remains unclear.

Methods

We retrospectively analyzed 296 patients with hypertension and prostate cancer from May 1, 2012, to October 1, 2015. The overall survival was evaluated by Cox regression models and Kaplan-Meier analysis. In addition, a nomogram was established, and the accuracy of the model was assessed by a calibration curve.

Results

A total of 101 (34.1%) patients died. In the multivariate analysis, being Ki-67(+) was associated with a >5-fold increase in the risk of death (hazard ratio [HR] 5.83, 95% confidence interval [CI] 3.35-10.14, $p < 0.001$) and a 2-fold increase in the risk of progression (HR 2.06, 95% CI 1.37-3.10, $p < 0.001$). Multivariate Lasso regression showed that smoking, heart failure, ACS, Ki-67 expression, serum albumin, prognostic nutritional index, surgery, Gealson score, and stage were positively associated with prognosis in patients with prostate cancer. To quantify the contribution of each covariate to the prognosis, a nomogram of the Cox model was generated. The nomogram demonstrated excellent accuracy in estimating the risk of death, with a bootstrap-corrected C index of 0.829. There was also a suitable calibration curve for risk estimation.

Conclusions

The presence of Ki-67 predicts worsened outcomes for overall mortality. A cross-validated multivariate score including Ki-67 had excellent concordance and efficacy for predicting prostate cancer.

Background

Prostate cancer is the second most common malignancy, with high mortality among males.[1] In 2018, the morbidity and mortality of prostate cancer ranked fourth worldwide for the various forms of cancer, and the mortality is higher for patients with hypertension. Approximately 1.3 million new cases of prostate cancer were diagnosed across the globe in 2018, with 360,000 prostate cancer-related deaths.[2] In the United States, an estimated 12.9% of men will be diagnosed with prostate cancer during their lifetime, which seriously endangers men's health.[3] In recent years, the mortality of prostate cancer has declined in many countries because of earlier diagnosis and improved treatment.[4] Nevertheless, the

clinical course of prostate cancer is highly variable, and predicting prognosis and recurrence is still a problem worthy of our attention.

With the development of immunohistochemistry and various specific antigens, many difficult tumors can be diagnosed definitely. Malignant tumor development is closely related to the state of cell proliferation. The indicators for assessing proliferation status, such as proliferation-related nuclear antigens and the mitotic index, can provide useful information about tumor biology and offer guidance in postoperative chemotherapy regimens.[5] Ki-67, also known as MKI67, is a nuclear protein involved in regulation of the cell cycle and cell proliferation, which is closely related to mitosis. Immunostaining with monoclonal antibody against Ki-67 is one of the most reliable markers for rapidly determining the growth fraction of neoplastic human cell populations[6, 7] and reflects the tumor cell proliferation rate. The technique is widely used in routine clinical pathology because of its simple detection technology and important pathological significance.[8] Previous research has shown that Ki-67 is strongly associated with metastasis, aggressiveness, and prognosis in many different malignancies, especially breast cancer.[9-12] However, due to conflicting results and limited sample sizes, the prognostic value of the Ki-67 index in prostate cancer is still debated.

To explore the role of Ki-67 in the prognosis of patients with prostate cancer, we launched a real-world study in the Chinese population. The prognostic value of Ki-67 was determined via multivariate-adjusted survival analyses. Subsequently, a multivariate score model including Ki-67 status was established to predict the prognosis in these patients.

Methods

Patients and study design

We retrospectively reviewed a total of 323 consecutive patients with prostate cancer who were hospitalized from May 1, 2012, to October 1, 2015, in the First Affiliated Hospital of Soochow University and The Ninth People's Hospital of Suzhou according to the patient journals and biobank in these hospitals. The patients were all diagnosed with prostate cancer via histological assessment of ultrasound-guided biopsy and were identified from patient journals and data collected from the biobank. Patients with severe renal or hepatic dysfunction, or withdrawing treatment, and those without follow-up information or lacking information on Ki-67 were excluded (Figure 1). Finally, 296 patients were analyzed in this study. Patient age, body mass index, serum total prostate-specific antigen (PSA), serum free PSA, serum albumin, neutrophil and lymphocyte count, neutrophil-lymphocyte ratio, level of hemoglobin, platelet count, prognostic nutritional index, intraoperative or postoperative bleeding, alcohol use, history of smoking, heart failure, acute coronary syndrome, hypertension, diabetes, hyperlipemia, TNM stage, risk stage, history of surgery, hormone therapy, Karnofsky Performance Status, Gleason grouping, Zubrod-ECOG-WHO score, and chemoradiotherapy status were recorded. The median follow-up was 60.0 months. Written and informed consent was obtained from all patients or their immediate family members. All

protocols conformed to the guidelines with the ethics committee of Soochow University and in accordance with the Declaration of Helsinki. The data extraction was conducted by two independent investigators using a pre-designed data extraction form. Divergences, especially in operation notes, were resolved by consensus or by consulting two senior investigators (Jun Ouyang and Jianchun Chen).

PSA evaluation and morbidity

PSA was estimated and urinary morbidity (pollakiuria/urgency, difficulty urinating, urinary incontinence, and mission pain) recorded according to the National Cancer Institute common terminology criteria for adverse events version 3.0 (NCI-CTCAE v3.0) at each regular visit to the urologist as part of routine care for the prostate cancer patient.

Immunohistochemical assessment

Representative paraffin-embedded tumor tissue blocks were cut into five micro slides. Immunohistochemical analysis was performed on each tumor slide block for Ki-67 using standard streptavidin ABC methodology. Antigen retrieval was performed by pressure cooking at pH 6.0 for 2 min (BioGenex Inc., San Ramon, CA, USA).

An immunohistochemical assay with tumor markers was performed using the antibodies and conditions. Sections (5 μ m) were cut from formalin or 50% alcohol-fixed paraffin-embedded cell blocks and stained with Ki-67 (clone MIB1, 1:160 dilution; DAKO, Carpinteria, Calif., USA), using tonsillar tissue as the positive control. Negative controls with the primary antibody replaced with a buffer were run simultaneously. Antigen retrieval was performed in citrate buffer at pH 6 under pressure for 3 min. The Envision Dual Link Kit (DAKO) was used for detection, with diaminobenzidine as the chromogen and hematoxylin as the counterstain. Staining was considered positive when nuclear positivity was observed. The Ki-67 labeling indices of the eight corresponding resections were also determined by image analysis. Finally, we calculated the relative expression of Ki-67, with expression >5% considered positive (+).

Sample size assessment

We retrospectively reviewed a total of 157 consecutive patients with prostate cancer who were hospitalized from Jan 1, 2010, to May 1, 2012, according to the patient journals and biobank in these hospitals. We used this data for the assessment. The sample size assessment was performed using the software NCSS-PASS version 11.0 (<https://www.ncss.com/software/pass/>). Power was set to 0.90 and alpha 0.5. The mortalities of the Ki-67(+) and Ki-67(-) groups (0.113 and 0.519) were inputted into the PASS. The actual hazard ratio was set as 2, and the minimum sample size was calculated to be 280 (control = 142, experiment = 138). In the prediction model, we selected 9 variants for the final model, and our sample was more than 10 times the number of variants. So, our sample size was acceptable and the sample size report was displayed in **Supplemental Material Part II**.

Statistical analysis

Continuous variates with normal and skewed distributions were presented as mean \pm standard deviation and medians with interquartile ranges and compared using the unpaired t-test and Mann-Whitney U test, respectively. Categorical variates were presented as percentages and compared using the κ^2 test. Cumulative incidence was visualized using the Kaplan-Meier curve and compared using the log-rank test. Univariate and multivariate survival analyses of overall mortality (OM) were performed using the Cox proportional hazard model. The importance of covariates to the prognosis was visualized using forest plots. Independent risk factors were used in least absolute shrinkage and selection operator (LASSO) regression, which is suitable for the regression of high-dimensional data, in order to select the most useful predictive features from the primary data set. A radiomics score (Rad-score) was calculated for each patient via a linear combination of select features that were weighted by their respective coefficients.

A nomogram was obtained based on the results of the multivariate Cox regression analysis using the 'rms' package of R, version 3.6.0 (<http://www.r-project.org/>). The nomogram is based on proportionally converting each regression coefficient in the multivariate Cox regression to a 0 to 100-point scale. To use the nomogram, find the position of each variable on the corresponding axis, draw a line to the points axis for the number of points, add the points from all variables, and then draw a line from the total points axis to determine the overall survival (OS) probabilities at the lower line of the nomogram. The effect of the variable with the highest β coefficient (absolute value) is assigned 100 points. The points are added across independent variables to derive total points, which are converted to predicted probabilities. The predictive performance of the nomogram was measured by the concordance index (C-index) and calibrated with 1000 bootstrap samples to decrease the overfit bias.[13]

Results

The average age of the 296 patients with prostate cancer was 71.42 ± 7.99 years. The median follow-up of all patients was 60.0 months (IQR 50.0-75.3). The median serum total PSA was 32.91 (IQR 15.84-101.54). Among all patients, 116 (39%) had a history of alcohol use and 191 (65%) were smokers. Hypertension was present in approximately 46% of patients, and only 12 (4%) and 16 (5%) patients suffered from heart failure and acute coronary syndrome. Forty-six (16%) patients were diagnosed with diabetes. For therapies, 164 (55%) patients accepted the operation and 97 (33%) received hormone therapy. Only 40 (14%) patients received chemoradiotherapy. According to the immunohistochemical assessment, 153 (52%) patients were Ki-67(+). Compared to the Ki-67(-) group, the Ki-67(+) group had patients of higher age, BMI, and serum-free PSA ($p < 0.05$). In addition, the Ki-67(+) patients were more susceptible to hypertension (Table 1). In our retrospective study, 101 (34.1%) patients died; only 6 died from other reasons (2 from post-surgical bleeding, 3 from infection, and 1 from disseminated intravascular coagulation). Eighty-four (54.9%) patients in the Ki-67(+) group died, whereas 17 (11.9%) patients in the Ki-67(-) group died.

In univariate analysis, Ki-67-positivity was a strong predictor of both OM (hazard ratio [HR] 5.84, 95% confidence interval [CI] 3.46–9.84, $p < 0.001$; Table 2) and progression-free survival (PFS; HR 2.80, 95% CI

1.82–4.32, $p < 0.001$; Table 3). When adjusted for alcohol consumption, smoking, and age, Ki-67-positivity was a strong predictor of OM (HR 6.27, 95% CI 3.70–10.63, $p < 0.001$) (Table 2). Kaplan-Meier curves show that Ki-67(+) patients had an increased cumulative incidence of death compared to the Ki-67(-) patients (log-rank $p < 0.001$; Fig. 2A). In addition, Ki-67(-) patients had higher PSF compared to Ki-67(+) patients (Fig. 2B). After multivariate adjustment of the significant variants ($p < 0.05$), Ki-67-positivity was associated with a > 5-fold increase in the risk of death (HR 5.83, 95% CI 3.35–10.14, $p < 0.001$; Fig. 3) and 2-fold increase in the risk of progression (HR 2.06, 95% CI 1.37–3.10, $p < 0.001$; Fig. 4).

As an important biomarker of prostate cancer, Ki-67 expression has significant prognostic value for multiple outcomes. Successful prediction may promote prophylaxis for progression and is proposed to improve the prognosis of prostate cancer. Therefore, we modeled a multivariate risk score for the prognosis of prostate cancer. Univariate analyses yielded age, alcohol use, smoking, heart failure, ACS, hypertension, Ki-67 expression, StPSA, SfPSA, serum albumin, lymphocyte count, hemoglobin level, prognostic nutritional index, surgery, KPS, Gealson score, ZPS, TNM, and risk stage as risk factors for death (Table 2). Putting all significant variables ($p < 0.05$) above into the multivariate cox regression, smoking, heart failure, ACS, Ki-67 expression, serum albumin, prognostic nutritional index, surgery, Gealson score, and stage were the independent risk factors for death in patients with prostate cancer (Fig. 3), but the HR was highest for Ki-67-positivity (HR 5.83, 95% CI 3.35–10.14, $p < 0.001$). Next, we put all of the independent risk factors into multivariate LASSO regression, which showed that the suitable number of variants was nine. Thus, smoking, heart failure, ACS, Ki-67 expression, serum albumin, prognostic nutritional index, surgery, Gealson score, and stage were selected in the predictive model. To quantify the contribution of each covariate to the prognosis of prostate cancer, a nomogram of the Cox regression model was generated (Fig. 5A). The resulting model was internally validated using the bootstrap validation method. The nomogram demonstrated excellent accuracy in estimating the prognosis of patients with prostate cancer, with an unadjusted C index of 0.829 and bootstrap-corrected C index of 0.829. There was also a suitable calibration curve for risk estimation (Fig. 5B).

Discussion

This real-world study demonstrated the prognostic value of Ki-67 in patients with prostate cancer. In our analysis, smoking, heart failure, ACS, Ki-67 expression, serum albumin, prognostic nutritional index, surgery, Gealson score, and stage were associated with a significantly worsened prognosis for OS in prostate cancer patients. The multivariate analysis also indicated that Ki-67 was an independent risk factor for favorable OS.

☒ The occurrence of cancer is regarded as a result of acquired genetic changes that regulate signal transduction pathways involved in cell proliferation and cell cycle control, especially from G1 to S phase. [14] Studies of cell proliferation are important for the invasiveness and prognosis of various malignancies.[15] With the accumulation of genetic alterations in dysplastic lesions of carcinogenesis, cellular proliferation becomes out of control.[16] When cells are in a state of uncontrolled proliferation, a series of malignant biological behaviors occur, including abnormal DNA structure and/or function,

accelerated transcription and translation during protein biosynthesis, and uncontrolled cancer cell growth. [17] A large number of studies have shown that tumor proliferation is a significant marker of poor prognosis in prostate cancer with a potential for routine application. [18–20] Ki-67 is one of the markers worth studying. [21]

Ki-67 is a nuclear protein involved in ribosomal RNA synthesis and important for the cell cycle. [22] Previous research has shown that Ki-67 contains an FHA domain at its amino terminus, which has an important role in the regulatory pathways involving Ser/Thr phosphorylation. This is similar to the structure of other proteins involved in cell cycle regulation. [23] Another FHA binding partner of Ki-67, NIFK, is considered to promote cell proliferation and cancer metastasis. [24, 25] Ki-67 is expressed at all stages of the cell cycle except G0 phase. [26] Consequently, Ki-67 has been widely used in histopathology to detect cycling cells to measure the growth fraction in the tissue. [27] Many studies have indicated that Ki-67 has high prognostic significance for various types of cancer and is related to tumor aggression, vascular invasion, and tumor metastasis. [28, 29]

In breast cancer, Ki-67 has been shown to be useful for assessing the prognosis of patients. [30] Although up-regulated Ki-67 has been confirmed as a significant marker of poor prognosis in prostate cancer. [9, 31, 32] For example, Berlin et al. found that Ki-67 is associated with prognosis in localized prostate cancer after treatment. [33] Richardsen et al. found that Ki-67 expression is higher in metastatic prostate cancer than non-metastatic prostate cancer. [34] Our study also indicated that Ki-67-positivity is a strong predictor of both OM and PFS. However, other researchers have found no significant correlation between the expression of Ki-67 and prostate cancer. [6, 35, 36] However, Ki-67 is controversial due to the lack of standardization in immunohistochemical assays, quantification methods, cutoff-points used for risk classification, and biological tumor heterogeneity. [37] For these and other reasons, Ki-67 has not yet been implemented to assess the prognosis of prostate cancer. Therefore, the prognostic value of Ki-67 in prostate cancer requires extensive prospective studies.

Our study also observed a correlation between Ki-67 and age, hypertension, and some other factors. Some studies have shown that the proliferation activity of prostate cells increases with age in male patients, which indicates that the high expression of Ki-67 is a systematic age-related phenomenon. [38, 39] This is in agreement with the results of our study, but some other studies have not found that Ki-67 is significantly associated with age. [40] This may be due to the differences in age distribution and racial differences in the population.

Hypertension and ACS are pathological conditions that damage the endothelium, triggering cell proliferation, vascular remodeling, and other malignant biological behaviors. [41, 42] A previous study considered that the expression of Ki-67 was lower in smokers than non-smokers, [43] but the mechanisms underlying this are still unclear. A meta-analysis reported that low Ki-67 is associated with a lower risk of developing diabetes mellitus, [33] but our study did not find an association between Ki-67 and diabetes mellitus.

Strengths And Limitations

A strength of our study was the large sample size in the Chinese population. This could give doctors a reference for the treatment of prostate cancer. These advantages may facilitate drawing a consistent conclusion within a dedicated ethnic group. However, the data came from the 2012–2015 period, and even the most recent patients were at least 4 years ago. We will use these latest data on prostate cancer patients in the future. In addition, the treatments were different and subgroup analysis may be needed after enrolling more patients. Bias may also be difficult to avoid because management of the patients in 2012 may be different from management in 2015.

Conclusions

In conclusion, our investigation found that the presence of Ki-67 is a strong predictor of both OM and PFS, making it an independent biological marker for predicting the prognosis of prostate cancer patients with hypertension. However, the mechanism of Ki-67 in prostate cancer is still unclear, and further investigation is needed.

Declarations

Ethics approval and consent to participate

This study was approved by the Committee for the Ethical Review of Research at the First Affiliated Hospital of Soochow University, and was conducted in accordance with institutional guidelines and the Declaration of Helsinki. Informed consent was obtained from all patients prior to data collection.

Consent to publish

We obtained consent from all authors for publication.

Availability of data and materials

All the data and materials are available if necessary.

Competing interests

The authors declare no competing financial interests.

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Authors' Contributions

ZC designed and performed research studies, analyzed the data, and wrote the manuscript. MX and ZZ performed research studies and analyzed the data. MJ and JZ contributed to the collection and analysis of clinical data. GS and YZ contributed to the data analysis. JC and JO contributed to the research design, data analysis, writing the manuscript, and supervision of the study.

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Not applicable.

Abbreviations

HF, heart failure; ACS, acute coronary syndrome; BMI: body mass index; Hb: hemoglobin; PLT: platelet; KPS: Karnofsky Performance Status; ZPS: Zubrod-ECOG-WHO; NLR: neutrophil lymphocyte ratio; HR: hazard risk; OS: overall survival; PFS: progression-free survival; OM: overall mortality.

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Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

Figures

Figure 1

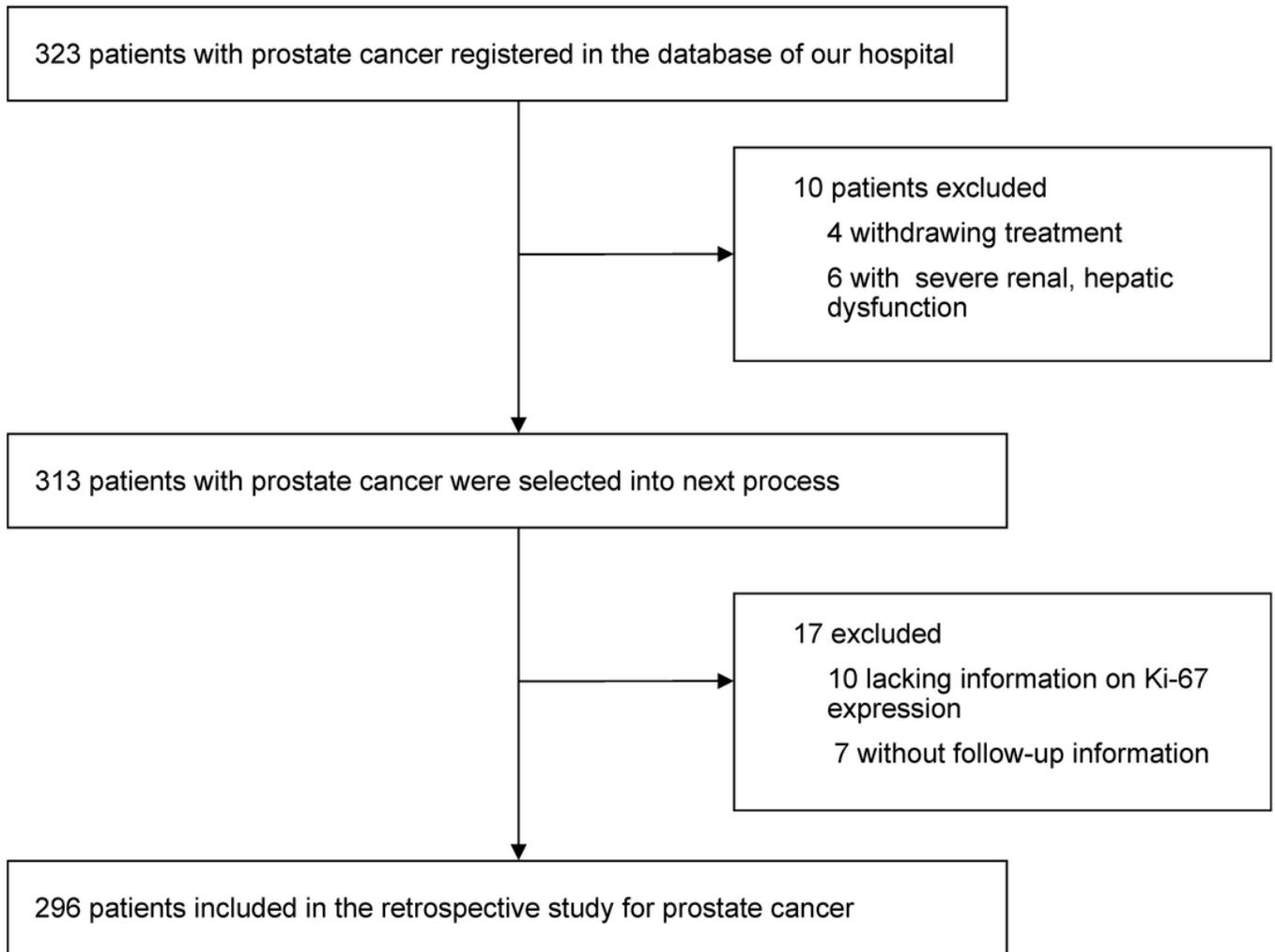
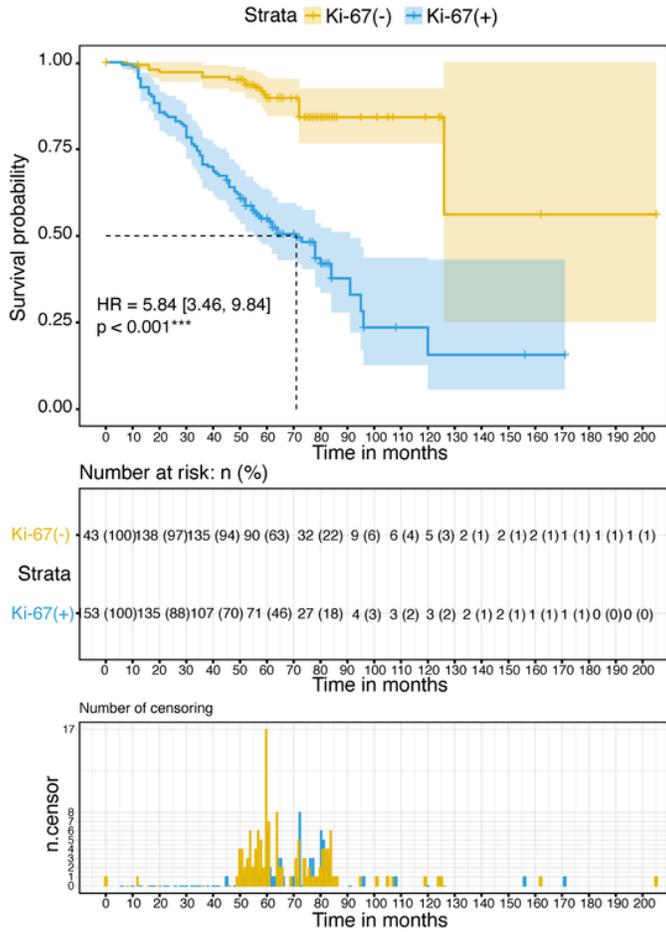


Figure 1

Flow-chart of patient selection.

Figure 2

A



B

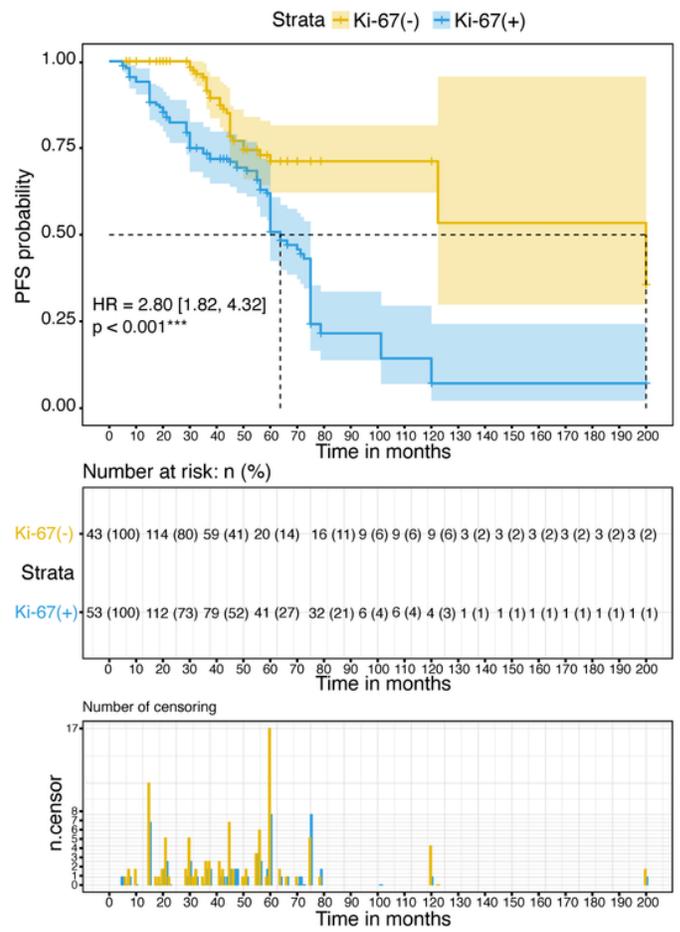


Figure 2

Multivariate analysis of survival in patients with prostate cancer. A. Unadjusted Kaplan-Meier curve of OS in Ki-67-positive vs. Ki-67-negative patients. B. Unadjusted Kaplan-Meier curve of PFS in Ki-67(+) vs. Ki-67(-) patients. ***p<0.001.

Figure 3

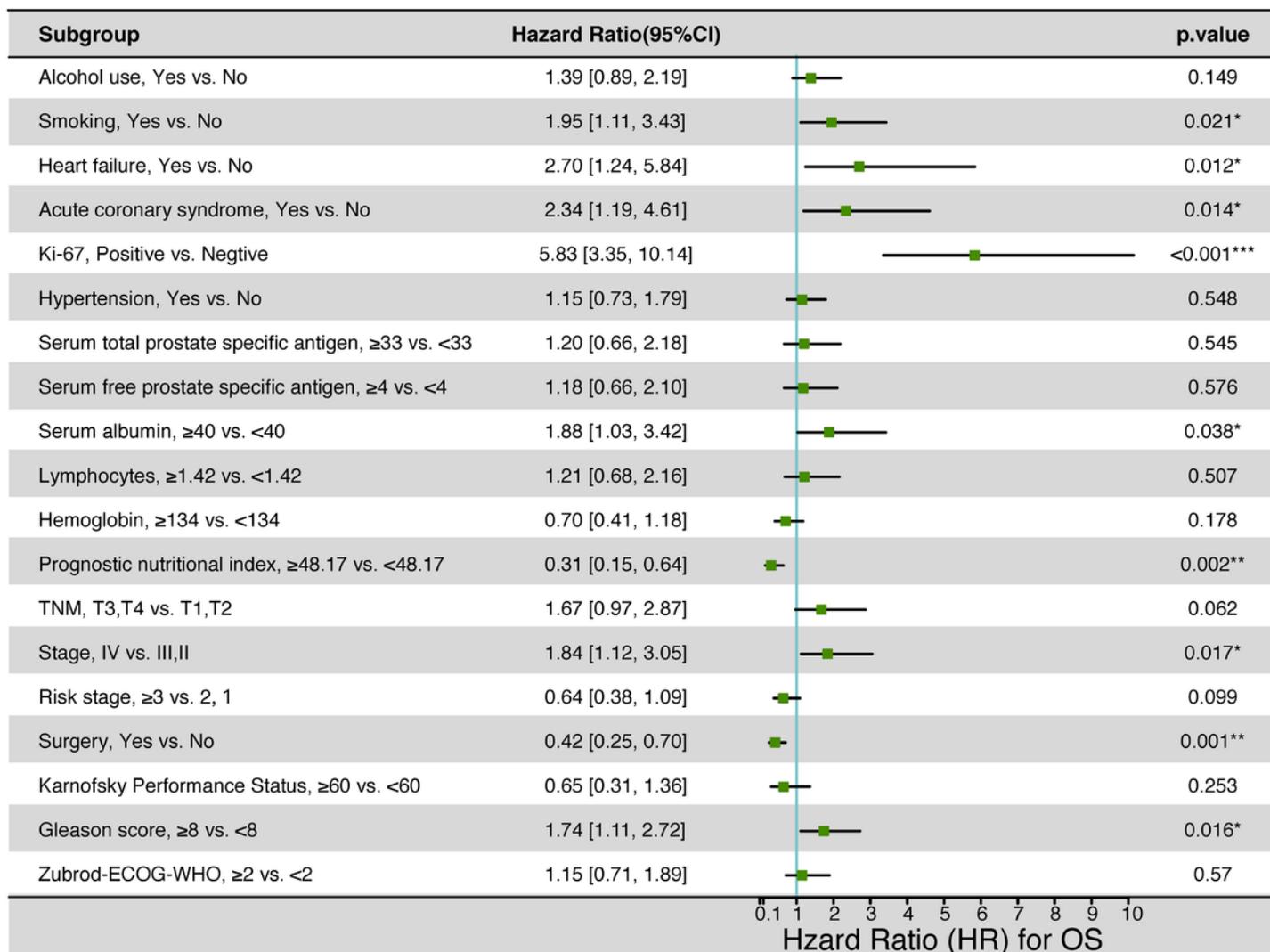


Figure 3

Forest plot for OS in patients with prostate cancer. Hazard ratios are established by the Cox regression model. ***p<0.001, **p<0.01, *p<0.05.

Figure 4

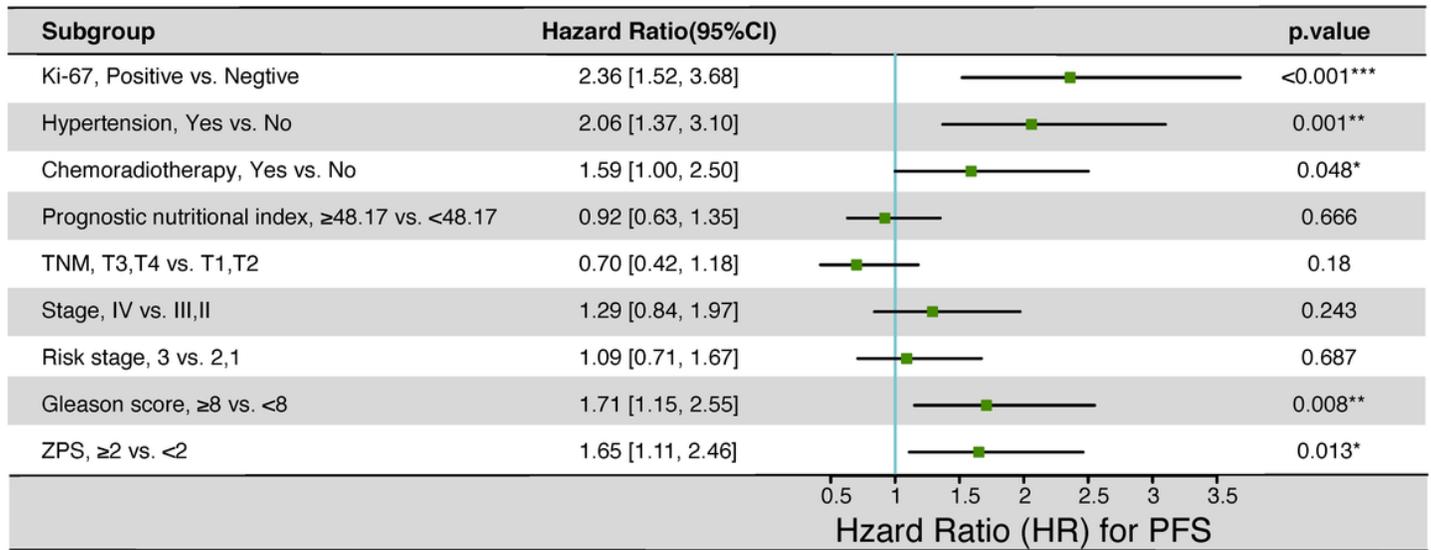
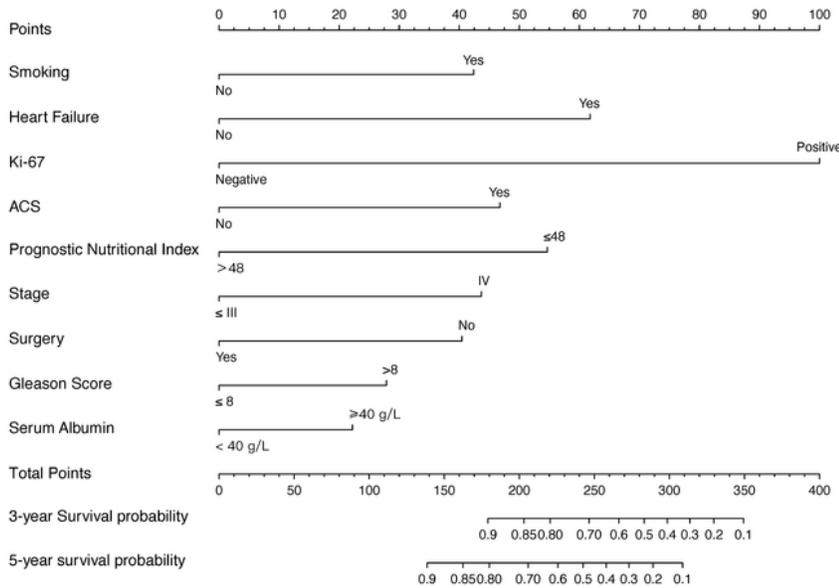


Figure 4

Forest plot for PFS in patients with prostate cancer. Hazard ratios are established by the Cox regression model. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Figure 5

A



B

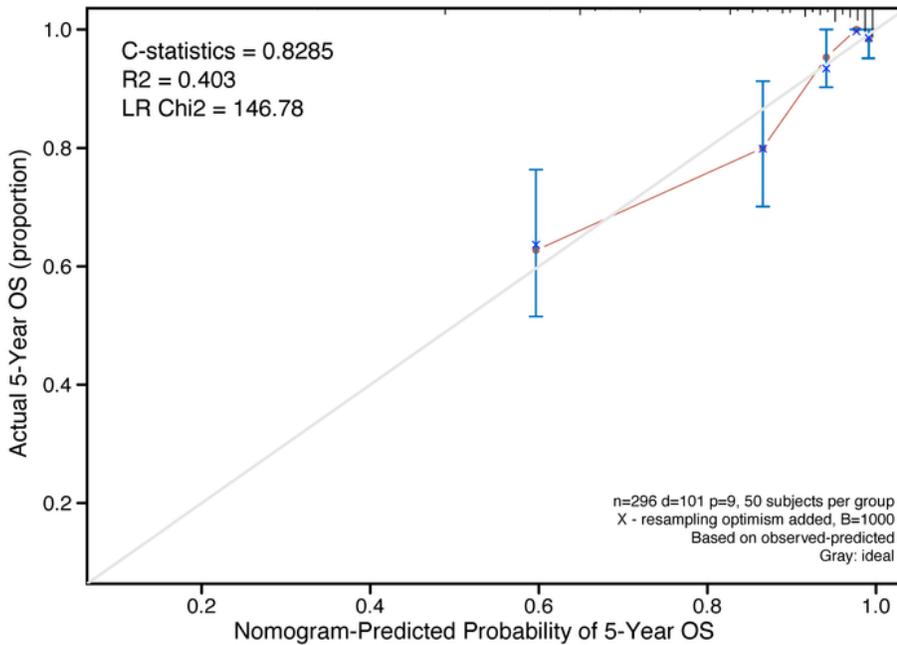


Figure 5

Nomogram estimation of the prognosis of prostate cancer. A. Nomogram estimating the prognosis of patients with prostate cancer in different variations. B. Validity of the predictive performance of the nomogram in estimating the prognosis of patients with prostate cancer.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Patientsdataforsamplesizeassessment.xlsx](#)
- [Table1.xlsx](#)
- [Table3.xlsx](#)
- [SupplementMaterialsv3.pdf](#)
- [Table2.xlsx](#)