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TITLE PAGE

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USE OF ^{18}F-NaF-PET IN THE STAGING OF SKELETAL METASTASES OF NEWLY DIAGNOSED, HIGH-RISK PROSTATE CANCER PATIENTS - A NATIONWIDE COHORT STUDY.

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ABSTRACT

Objective To determine whether preoperative staging of high-risk prostate cancer with ^{18}F-NaF PET reduces the risk of skeletal metastases.

Design Nationwide, population-based cohort study using real-world data.

Setting The study used national health registries, including all sites in Denmark from 2011-2018.

Participants Newly diagnosed high-risk prostate cancer patients who underwent radical prostatectomy from 2011-2018. Patients were stratified into two groups according to the preoperative imaging modality of either ^{18}F-NaF PET or bone scintigraphy.

Main outcome measures The risk of skeletal-related events as a proxy for skeletal metastases following radical prostatectomy. The secondary endpoint was overall survival.

Results Between January 1, 2011, and December 31, 2018, 4,183 high-risk patients underwent radical prostatectomy. Of these patients, 807 (19.3%) underwent ^{18}F-NaF PET and 2,161 (51.7%) underwent bone scintigraphy. The remaining 30% were examined by a different imaging method or did not undergo imaging. Using the inverse probability of treatment weighting to control potential confounding, the hazard ratio of experiencing a skeletal-related event for patients in the ^{18}F-NaF PET group versus the bone scintigraphy group was 1.15 (95% CI 0.86-1.54). The 3-year survival rates were 97.4% (95% CI 96.1-98.7) and 97.1% (95% CI 96.4-97.9) for patients receiving ^{18}F-NaF PET and bone scintigraphy, respectively.

Conclusion High-risk prostate cancer patients undergoing preoperative staging with ^{18}F-NaF PET did not display a lower risk of developing skeletal-related events after prostatectomy compared to patients undergoing bone scintigraphy. The survival rates were similar between the two groups. The results of this study support the existing guidelines that recommend bone scintigraphy as the first choice in the primary staging of prostate cancer.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study identified a cohort from all institutions in Denmark using high-quality registry data.
- The study uses routinely collected health data that is not specifically collected for the purposes of this research, resulting in a minor degree of missing data.
- Regression analysis weighted by the inverse probability of treatment ensured consideration of all measured confounders and addressed confounding by indication.
- This large cohort study using real-world data provides the first evidence that there is no clinical benefit of 18F-NaF PET in terms of patient-relevant outcomes.

INTRODUCTION

Prostate cancer is one of the most common malignancies in the Western world, with over 1.4 million new cases reported in 2020.[1] Prostate cancer frequently metastasizes to the bone, which is associated with significant morbidity and mortality.[2 3] Accurate detection of bone metastases at primary staging is essential for decision-making regarding subsequent management. At the time of diagnosis, the risk of recurrence is determined based on the PSA level, Gleason score, and clinical tumour stage (T-stage).[4] Patients classified as unfavorable–intermediate risk or high risk will often receive preoperative staging by imaging. International urology and oncology guidelines recommend bone scintigraphy with 99mTechnetium-labeled phosphonate (99mTc) for the assessment of bone metastases at primary staging.[4 5]

However, several studies have shown that the bone-specific positron emission tomography (PET) tracer ^{18}F-sodium-fluoride (^{18}F-NaF) is superior to bone scintigraphy in terms of its diagnostic accuracy for detecting bone metastases including fewer equivocal findings.[6-8] In previous studies, the sensitivity of bone scintigraphy for the detection of bone metastases varied from 57% to 97%, and the specificity varied from 57 to 80%.[6-9] In contrast, the sensitivity of ^{18}F-NaF PET for the diagnosis of bone metastases has ranged from 81 to 100% in the majority of studies, with a specificity ranging from 71 to 100%.[6-8 10 11] With the purported lower accuracy of bone scintigraphy, the risk of misdiagnosing patients is high, possibly resulting in suboptimal treatment strategies. Among patients referred for suspected metastases, the use of ^{18}F-NaF PET instead of bone scintigraphy in patients with prostate cancer has been shown to affect the patient management strategy in 6-12% of cases.[12 13] However, no studies have documented that the subsequent change in patient management strategies induced by ^{18}F-NaF PET and its improved diagnostic accuracy confer any patient benefit in terms of mortality, morbidity and quality of life. Thus, we performed a cohort study with real-world data of men diagnosed with prostate cancer in Denmark who underwent either bone scintigraphy or ^{18}F-NaF PET as part of primary staging before curative intent prostatectomy to examine whether the type of preoperative imaging modality was associated with overall survival and skeletal-related events (SREs) after radical prostatectomy.

METHODS

Study Population and Data Sources

This nationwide register-based cohort study was conducted in Denmark, which has approximately 5.8 million residents. In Denmark, all residents are provided with free, tax-supported health care by the National Health Service. A unique 10-digit civil registration number is assigned to all residents at birth by the Central Office of Civil Registration. This number allows unambiguous linkage across all Danish population-based registries.[14] Reporting to the registries by clinicians is mandatory, which ensures high completeness of medical information. The applied data included nationwide information from the Danish Cancer Registry,[15] the Civil Registration System,[16] the Danish National Patient Registry,[17] the Register of Laboratory Results for Research,[18] the Danish Prostate Cancer Database,[19] the Danish National Pathology Register,[20] and the Register of Causes of Death.[21] Appendix 1 (p 1) provides a detailed description of the codes found in the registries for prostate cancer characteristics, treatment, outcomes, and covariates. Furthermore, the study is reported in accordance with STROBE guidelines, and a checklist is provided in the supplementary files.

Identifying Men with Prostate Cancer

No formal screening program for prostate cancer existed during the study period. Therefore, men were referred to the urology department upon suspicion of prostate cancer. We used the Danish National Patient Registry to identify a cohort consisting of men with a first-time prostate cancer diagnosis from 2011 through 2018 who had undergone radical prostatectomy. This registry was established in 1977 for hospitalized patients; outpatient visits at hospitals have been included since 1995.[17] The registry includes dates of admission and discharge, diagnosis (ICD-10 codes), surgical procedures, and treatment information. The validity of a prostate cancer diagnosis in this register has previously been evaluated and found to be high, with a positive predictive value of nearly 90%.[22]

Risk Classification

We restricted the cohort to patients we could classify as having a preoperative high risk of cancer recurrence according to the European Association of Urology (EAU) risk classification of prostate cancer. The EAU defines high-risk patients as those with a PSA of more than 20 ng/mL OR a Gleason score >7 OR a T-stage of T2c as the minimum.[4] PSA values were retrieved from the Danish Register of Laboratory Results, which includes laboratory data from four of the five regions of Denmark.[18] Data from the last region were obtained directly from the relevant regional database. The Gleason score was obtained from the Pathology Register, which contains information on all pathological examinations conducted in Denmark since 1997. T-stage was obtained from the Danish Cancer Registry, which has prospectively recorded all cancers diagnosed in Denmark since 1943, classified according to ICD-10, and ICD Oncology codes (ICD-0-3) for topography and morphology.[15] For all three variables, we included the latest recorded value within six months prior to surgery. If PSA, Gleason score, or T-stage were missing, we used the Danish Prostate Cancer Database to fill in the

missing variables. This register is a nationwide clinical cancer database established in 2010 that records data on all incident, historically verified prostate cancer cases.

Imaging Modality

We retrieved information on imaging modalities from the Danish National Patient Registry. We identified the preoperative use of bone scintigraphy and ^{18}F-NaF PET, recorded up to 6 months before surgery, combined with computer tomography (CT) or magnetic resonance imaging (MRI). Single-photon emission (SPECT)/CT was conducted according to institutional practices. Patients were categorized according to their preoperative imaging into two groups: those who underwent bone scintigraphy only (bone scintigraphy group) and those who underwent ^{18}F-NaF PET scan with or without bone scintigraphy (^{18}F-NaF PET group). In general, each site performed only one of the two scans; thus, physicians did not stratify patients according to a specific imaging modality. Patients with an ^{18}F-NaF PET scan performed as a part of a clinical research project were excluded from the cohort because the results of these scans were not made available to the referring physician.

SREs and Bone Metastases

We obtained information on SREs through the Danish National Patient Registry. SREs comprised the following events occurring after the date of radical prostatectomy: radiation to the bone defined as 1-4 treatments with external radiation therapy (standard practice in Denmark), pathological and osteoporotic fractures, spinal cord compression, surgery to the bone, or a first-time bone metastasis diagnosis code.

Mortality

Mortality and migration updates were obtained from the Civil Registration System, which is updated daily.[14] The register contains information on the vital status (dead or alive), date of death, and migration status of all Danish citizens.

Comorbidity

We used the Charlson comorbidity index to describe preexisting comorbidities in the prostate cancer cohort[23] (appendix 1 p 2). We calculated the index based on diagnoses recorded in the Danish National Patient Registry up to ten years before the date of surgery. For analysis, we categorized the index into 3 comorbidity levels, including 1) those without comorbidity, 2) those with a comorbidity index equal to 1, and 3) those with a comorbidity index above 1.

Statistical Analysis

Baseline characteristics are reported as frequencies with percentages and medians with interquartile ranges. We estimated the cumulative risk of SREs according to the type of imaging modality and plotted the cumulative risk as a function of time since radical prostatectomy; death was treated as a competing risk event. Patients contributed time at risk from the date of radical prostatectomy until the date of first-time registered

SRE, migration, death, or December 31, 2018, whichever came first. Finally, we similarly estimated the cumulative incidence of death.

For the main analysis, we used Cox proportional hazards regression analysis to estimate the age-adjusted and multivariate-adjusted hazard ratios (HRs) of SREs with 95% CIs, comparing those who underwent ^{18}F-NaF PET scans with those who underwent bone scintigraphy. Additionally, to better control potential confounding by indication, analysis of the inverse probability of treatment weighting (IPTW) was performed based on the propensity score for ^{18}F-NaF PET. Propensity scores were calculated using logistic regression with the inclusion of the same variables as in the adjusted Cox analysis. We adjusted for age, Charlson comorbidity index, PSA (categorical variable: <10, 10-20, >20 ng/mL), Gleason score (categorical variable: <7, 7, >7), and T-stage (categorical variable: T1, T2, T3+T4). Adjusting with categorical variables was deemed necessary due to outliers and the limited number of records available on the outer areas of the scales. Furthermore, we stratified the analysis by PSA, Gleason score, T-stage, and year of radical prostatectomy. In the stratified analysis, we only adjusted for age and Charlson comorbidity index. An adjusted HR of death was also calculated. No further analyses were performed for patients with other types of imaging or no imaging before surgery.

Several sensitivity analyses were performed to test the robustness of our findings, including an analysis restricted to the capitol region of Denmark and the reclassification of the exposure group to include patients with both scans. An additional regression analysis was performed with imputed data on the missing values of PSA, Gleason score, and T-stage using multiple imputations.[24]

Statistical software

Data management and analyses were conducted in R 4.0.3 using RStudio 2020 (RStudio, PBC, Boston, MA) with the following packages: heaven, data.table, Publish, survival, stringr, mitools, smcfcs and ipw.

Ethics Approval

The Danish Data Protection Agency approved the use of data for this study (reference number 2008-58-0028). Furthermore, the study was granted approval by the Danish Patient Safety Authority to collect laboratory data (reference numbers 3-3013-3183/1 and 31-1522-37). Ethics approval is not required for historical register-based studies in Denmark.

Patient and public involvement

This study was observational and based on data from routine healthcare records. No patients were directly involved in the study.

RESULTS

Between January 1, 2011, and December 31, 2018, 36,910 men were diagnosed with prostate cancer in Denmark, of whom 8,726 (23.6%) underwent radical prostatectomy (Figure 1). Among those who underwent radical prostatectomy, 4,183 patients (47.9%) were classified as high risk according to the EAU preoperative staging criteria. A total of 2,161 (51.7%) high-risk patients undergoing surgery were evaluated for skeletal metastasis with bone scintigraphy only, and 807 (19.3%) men were evaluated with ^{18}F-NaF PET. Information on the PSA values, Gleason score, and T-stage from the registries ensured nearly 90% completeness of the high-risk classification, resulting in a large study population for our analysis. A notable proportion of high-risk patients (28.5%) underwent different imaging modalities or no imaging to evaluate bone metastasis, and a small portion of patients (0.5%) were excluded because they underwent project-related imaging. The median age at the date of radical prostatectomy was 67 years (interquartile range, 62-70.1), and the median follow-up from surgery was 4.1 years (interquartile range, 2.4-6.0 years). In general, patients receiving ^{18}F-NaF PET had a higher PSA level, Gleason score, and T-stage at primary staging (Table 1).

SREs and Bone Metastases

The unadjusted one-year cumulative risk of SREs was 2.4% (95% CI 1.8-3.1) for men who underwent bone scintigraphy and 4.3% (95% CI 2.8-5.7) for those who underwent ^{18}F-NaF PET (Figure 2). The unadjusted 3-year cumulative risk of SREs was 7.2% (95% CI 6.0-8.3) for men undergoing bone scintigraphy and 11.9% (95% CI 9.4-14.4) for those undergoing ^{18}F-NaF PET. Of the 300 men with at least one SRE recorded during follow-up, 53.7% had radiation to bone recorded as their first event, 30.7% had a pathological or osteoporotic fracture, 6.3% had spinal cord compression, 6.3% had a code for bone metastases, and 3.0% had bone surgery. In the main analysis, we did not find that ^{18}F-NaF PET decreased the HR of experiencing SREs after surgery; in contrast, we observed a slightly increased HR, which was reduced when adjusting the model (adjusted HR, 1.22; 95% CI 0.93-1.61; Figure 3). When we used IPTW to control for potential confounding factors, the risk of experiencing an SRE was attenuated (IPTW adjusted HR, 1.15: 95% CI 0.86-1.54; Figure 3). Stratified analyses similarly demonstrated increased HRs for SREs in patients undergoing ^{18}F-NaF PET compared to those undergoing bone scintigraphy, except for patients with stage 2 disease and those with a Gleason score <7 (Figure 3).

Survival

Figure 4 shows the cumulative survival curves of the cohorts for up to 7 years of follow-up. The one-year survival was 99.4% (95% CI 99.0-99.7) in men who underwent bone scintigraphy and 99.5% (95% CI 98.9-100) in men who underwent ^{18}F-NaF PET, and the corresponding 3-year survival rates in the cohorts were 97.1% (95% CI 96.4-97.9) and 97.4% (95% CI 96.1-98.7), respectively. Adjusted analyses showed a modest reduction in mortality for patients who underwent ^{18}F-NaF PET (adjusted HR, 0.89; 95% CI 0.61-1.30).

Sensitivity Analysis

Restricting to patients from the capitol region yielded cumulative SRE risk estimates consistent with those of the main analysis (appendix 1 p 3). Similar to the main analysis, the cumulative risk of SREs was higher for men evaluated with ^{18}F-NaF PET than for those evaluated with bone scintigraphy. Adjusted analysis for the capitol region was also comparable to the main analysis (appendix 1 p 4) and did not suggest any added value of using ^{18}F-NaF PET.

Including patients with both bone scintigraphy and ^{18}F-NaF PET in the bone scintigraphy group or excluding them entirely yielded HRs similar to those of the main analysis. A final analysis with imputed values for PSA, Gleason score, and T-stage yielded HRs similar to those of the analysis without imputation.

DISCUSSION

Principal findings

In this nationwide cohort study of Danish patients with high-risk prostate cancer undergoing prostatectomy, we found that preoperative staging with ^{18}F-NaF PET did not reduce the risk of SREs compared to staging with bone scintigraphy, whereas a slight tendency towards a reduction in all-cause mortality was observed in the group undergoing ^{18}F-NaF PET. To the best of our knowledge, this is the first study to evaluate patient-relevant outcomes of using a PET-based method for primary staging.

Comparison with other studies

Prior studies on ^{18}F-NaF PET in prostate cancer have focused on its improvements in diagnostic accuracy compared to bone scintigraphy[6-8] or its impact on patient management.[12 13] The superior diagnostic performance of ^{18}F-NaF PET should presumably result in improved patient selection for curative and life-prolonging treatment, leading to improvements in patient-relevant outcomes. However, in this study, we did not observe any superiority over bone scintigraphy in terms of patient benefit among newly diagnosed, high-risk prostate cancer patients.

Evidence of patient-relevant outcomes is often reported from randomized controlled trials. Randomized trials are, however, not commonly conducted within the field of imaging, and it has previously been debated whether randomized trials are necessary to evaluate diagnostic procedures. In prostate cancer, only two randomized controlled trials have been published, employing PET in one arm and standard imaging in the other arm. One such trial confirmed the diagnostic superiority of PSMA PET/CT during primary staging[25], whereas the other trial focused on the changes in patient management based on fluciclovine PET/CT at the time of biochemical recurrence;[26] none of these trials were linked to patient-relevant outcomes.

Randomized trials have demonstrated the clinical benefit of PET within other types of cancers, such as haematological and lung cancers.[27] Fischer et al. compared preoperative staging with FDG PET/CT to conventional staging by CT in lung cancer patients and found that patients in the PET/CT group showed a reduction in both the total number of thoracotomies and the number of futile thoracotomies; however, they did not observe a decrease in overall mortality.[28] Similar results were reported for colorectal liver metastases, with one study finding that FDG PET led to a reduction in futile laparotomies in 1 of 6 patients.[29] It could be expected that the use of ^{18}F-NaF PET would reduce the number of "futile" prostatectomies in patients harboring bone metastases at the time of diagnosis, thereby reducing the incidence of SREs postoperatively. With recent trials demonstrating superior diagnostic properties of PSMA PET for primary staging in high-risk prostate cancer, its impact on treatment choice—and perhaps outcome—is likely to be greater than that of ^{18}F-NaF PET.

Strengths and limitations

The major strengths of our study are its national scale, large cohort, high-quality registry data, and complete follow-up. The registration of information related to prostate cancer diagnosis and radical prostatectomy, as well as variables defining the high-risk population, is thought to be practically complete because of a uniformly organized health care system where healthcare is free (tax-supported) and available to all residents.[30] Furthermore, a median follow-up time of 4.1 years is adequate for the purpose of evaluating bone metastases not captured by the imaging modality at primary staging; hence, only patients with a negative scan will undergo radical prostatectomy with curative intend in Denmark.

Nevertheless, our study has several limitations worth considering. The potential of confounding by indication was particularly concerning because of the observed higher values for PSA, Gleason score, and T-stage in the ^{18}F-NaF PET group; however, the indication of usage was the same for both scans. Moreover, the demographics of the groups might have been more alike if the International Society of Urological Pathology (ISUP) grading system was used for the Gleason score, which distinguishes between normal high-risk prostate cancer and very high-risk (ISUP grade 5) cancer cases. It was not possible to use the ISUP grading due to unavailability in some of the registers. Furthermore, confounding by indication is only an issue in hospitals that offer both bone scintigraphy and ^{18}F-NaF PET, which is highly uncommon in Denmark. Since sites only used one of the imaging modalities, physicians did not have to choose between the two, resulting in minimal selection bias. We attempted to control for confounding by using a propensity score-based inverse probability of treatment weighting, but we cannot rule out residual confounding due to misclassified or unmeasured prognostic factors.

In the present study, we defined SREs as either external radiation therapy, pathological or osteoporotic fractures, spinal cord compression, surgery to the bone, or a bone metastases code. It can be speculated that patients treated at a site using ^{18}F-NaF PET would undergo ^{18}F-NaF PET rather than bone scintigraphy in case of biochemical recurrence, thereby increasing the detection of bone metastases during follow-up. However, the risk of SREs was primarily driven by a high percentage of radiotherapy of bone or fracture cases, which are not related to ^{18}F-NaF PET. Information regarding bone metastases was noted in only 6.3% of SREs across the groups.

Conclusions

In conclusion, we found that the use of ^{18}F-NaF PET at primary staging did not improve patient-relevant outcomes in terms of a reduction in SREs compared to that with bone scintigraphy.

ACKNOWLEDGMENT SECTION

Author Contributions

AWM, LJP and HDZ conceived the study and contributed to the literature search. AWM and CTP had access to the data and carried out the data management and analysis. AWM, CTP and MN designed the graphs. AWM, LJP, HDZ, CTP, MN and MTP aided in the interpretation of results. AWM prepared the first draft of the paper. All authors contributed to critical revision and approval of the final draft of the paper.

Conflict of Interest Disclosure

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/ coi_disclosure.pdf and declare: one author received grants from Bayer and Novo Nordisk for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Data availability statement

Data is not available to other researchers due to Registry or institutional database of patients providing routinely collected data.

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FIGURE LEGENDS

(Figure 1-4 are attached as separate PDF files)

Figure 1: Study profile

Study cohort of 2,161 men undergoing presurgical imaging with bone scintigraphy and 807 men undergoing ^{18}F-NaF PET.

Figure 2: Unadjusted cumulative incidence of skeletal-related events (SREs)

The unadjusted cumulative incidence with 95% confidence intervals of SREs in men after undergoing radical prostatectomy. Death was treated as a competing event. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

Figure 3: Main analysis results

Hazard ratios for SREs following radical prostatectomy among patients undergoing 18F-NaF PET before surgery versus patients undergoing bone scintigraphy.

Figure 4: Unadjusted cumulative incidence of death

Unadjusted cumulative incidence of death with 95% confidence intervals for men with prostate cancer after undergoing radical prostatectomy, stratified by type of imaging modality. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

Table 1: Baseline patient characteristics by imaging modality

Characteristics on the day of surgery for men with high-risk prostate cancer from 2011 to 2018. Percentages may not add up to 100 due to rounding or missing data. *Top 3 comorbidities. Abbreviations: IQR, interquartile range; PSA, prostate specific antigen; T-stage, tumour stage.

TABLES

Table 1. Baseline patient characteristics by imaging modality

	Bone scintigraphy (n = 2,161)	^{18}F-NaF PET (n = 807)	All (n = 2,968)
Age (years, median (IQR))	66.3 (61.7, 69.7)	67.9 (62.9, 71.2)	66.7 (62.0, 70.1)
Year of surgery			
2011-2013	852 (39.4)	212 (26.3)	1,064 (35.8)
2014-2015	602 (27.9)	235 (29.1)	837 (28.2)
2016-2018	707 (32.7)	360 (44.6)	1,067 (36.0)
PSA (ng/mL)			
<10	955 (45.0)	263 (33.1)	1,218 (41.8)
10-20	642 (30.2)	292 (36.8)	934 (32.0)
>20	526 (24.8)	239 (30.1)	765 (26.2)
Gleason biopsy score			
<7	345 (16.2)	70 (8.8)	415 (14.2)
7	1225 (57.5)	469 (58.6)	1,694 (57.8)
>7	560 (26.3)	261 (32.6)	821 (28.0)
Clinical T-stage			
T1	259 (12.6)	50 (7.5)	309 (11.4)
T2	1260 (61.5)	241 (36.0)	1,501 (55.2)
T3-T4	529 (25.8)	378 (56.5)	907 (33.4)
Comorbidity*			
Cardiovascular diseases	118 (5.5)	52 (6.4)	170 (5.8)
Other malignancies	102 (4.7)	64 (7.9)	166 (5.6)
Diabetes	62 (2.9)	48 (6.0)	110 (3.7)
Charlson comorbidity index			
1	267 (12.4)	115 (14.3)	382 (12.9)
>1	203 (9.4)	107 (13.3)	310 (10.4)

Characteristics on the day of surgery for men with high-risk prostate cancer from 2011 to 2018. Percentages may not add up to 100 due to rounding or missing data. *Top 3 comorbidities. Abbreviations: IQR, interquartile range; PSA, prostate specific antigen; T-stage, tumour stage.

Figure 1: Study profile

Study cohort of 2,161 men undergoing presurgical imaging with bone scintigraphy and 807 men undergoing 18F-NaF PET.

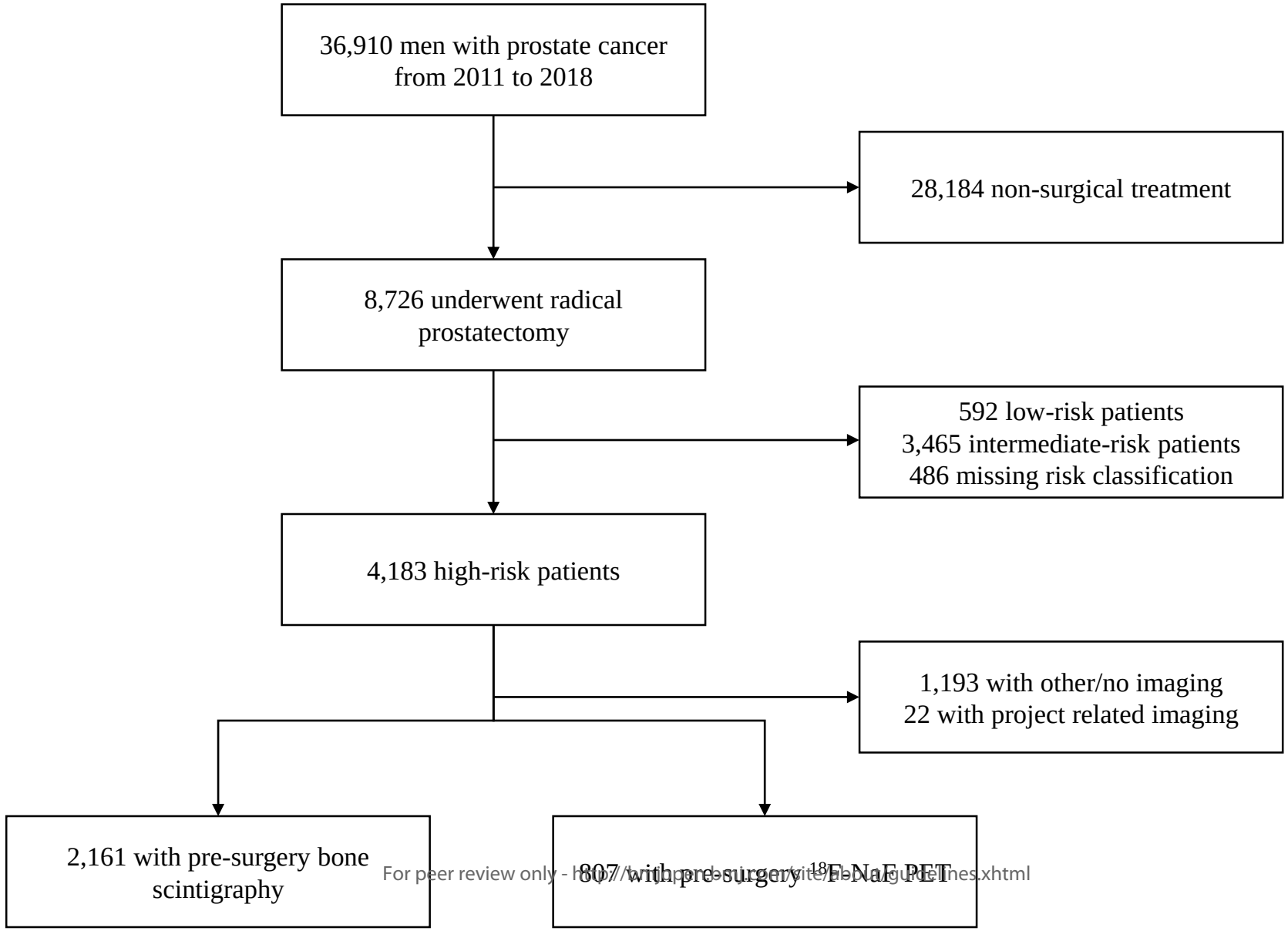


Figure 2. Unadjusted cumulative incidence of skeletal-related events (SREs)

The unadjusted cumulative incidence with 95% confidence intervals of SREs in men after undergoing radical prostatectomy. Death was treated as a competing event. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

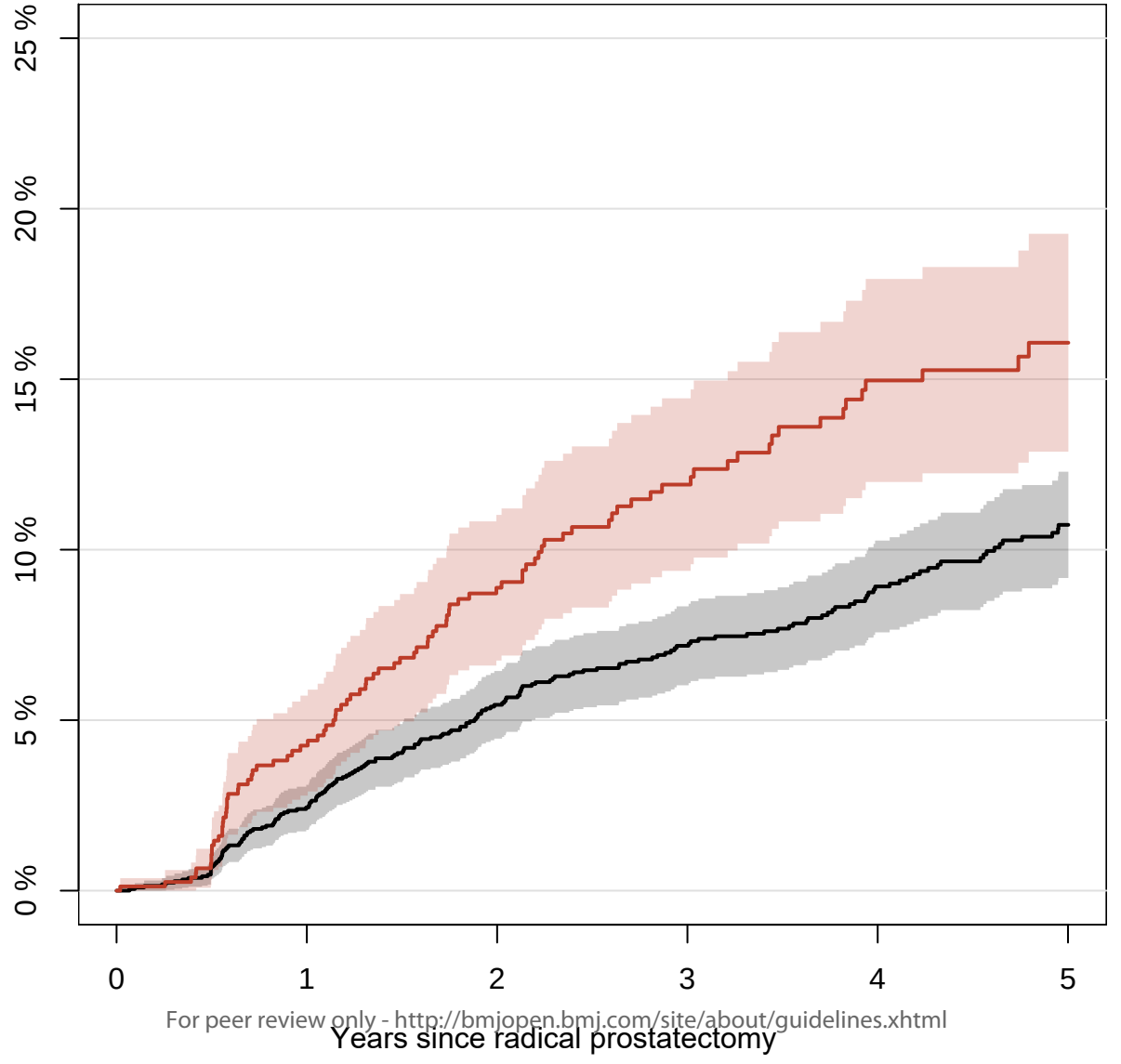


Figure 3. Main analysis results

Hazard ratios for SREs following radical prostatectomy among patients undergoing 18F-NaF PET before surgery versus patients undergoing bone scintigraphy.

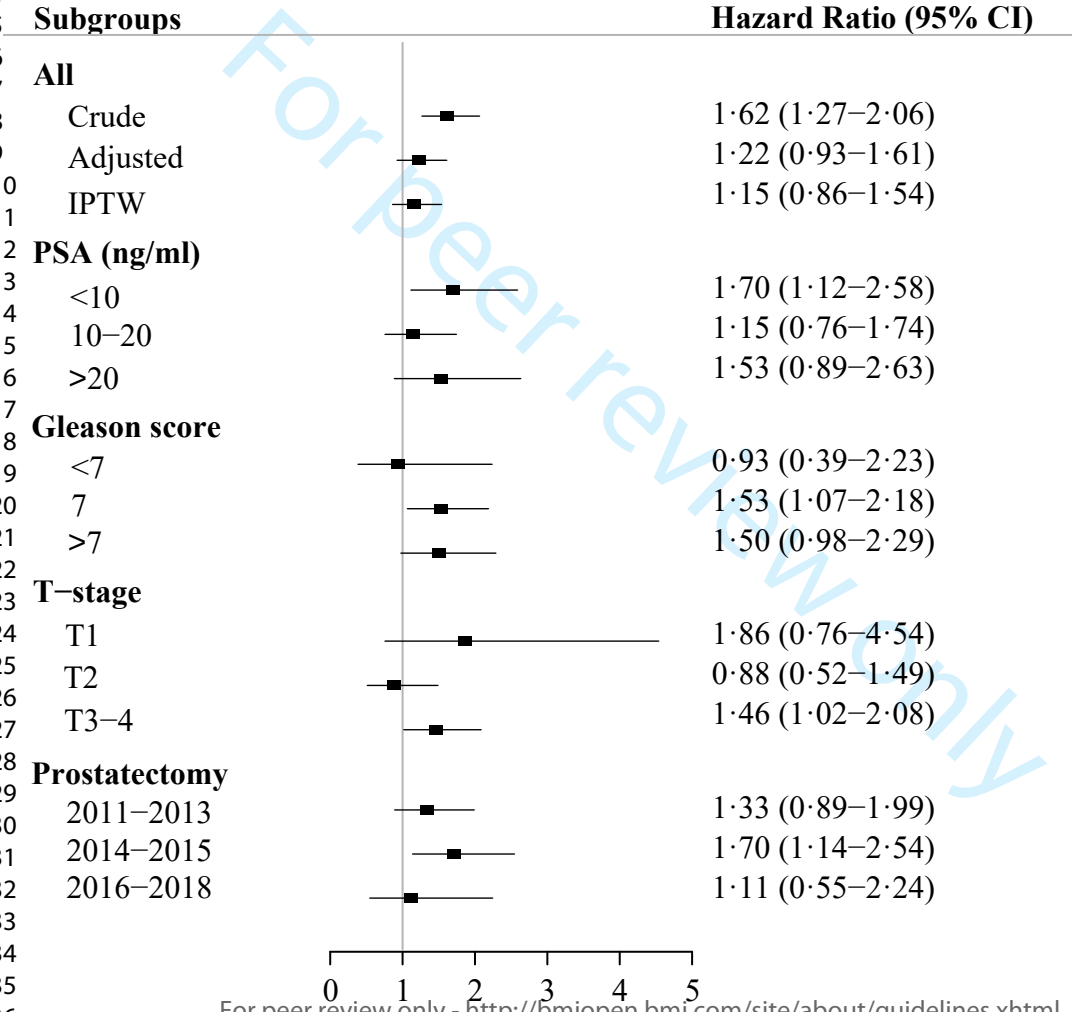
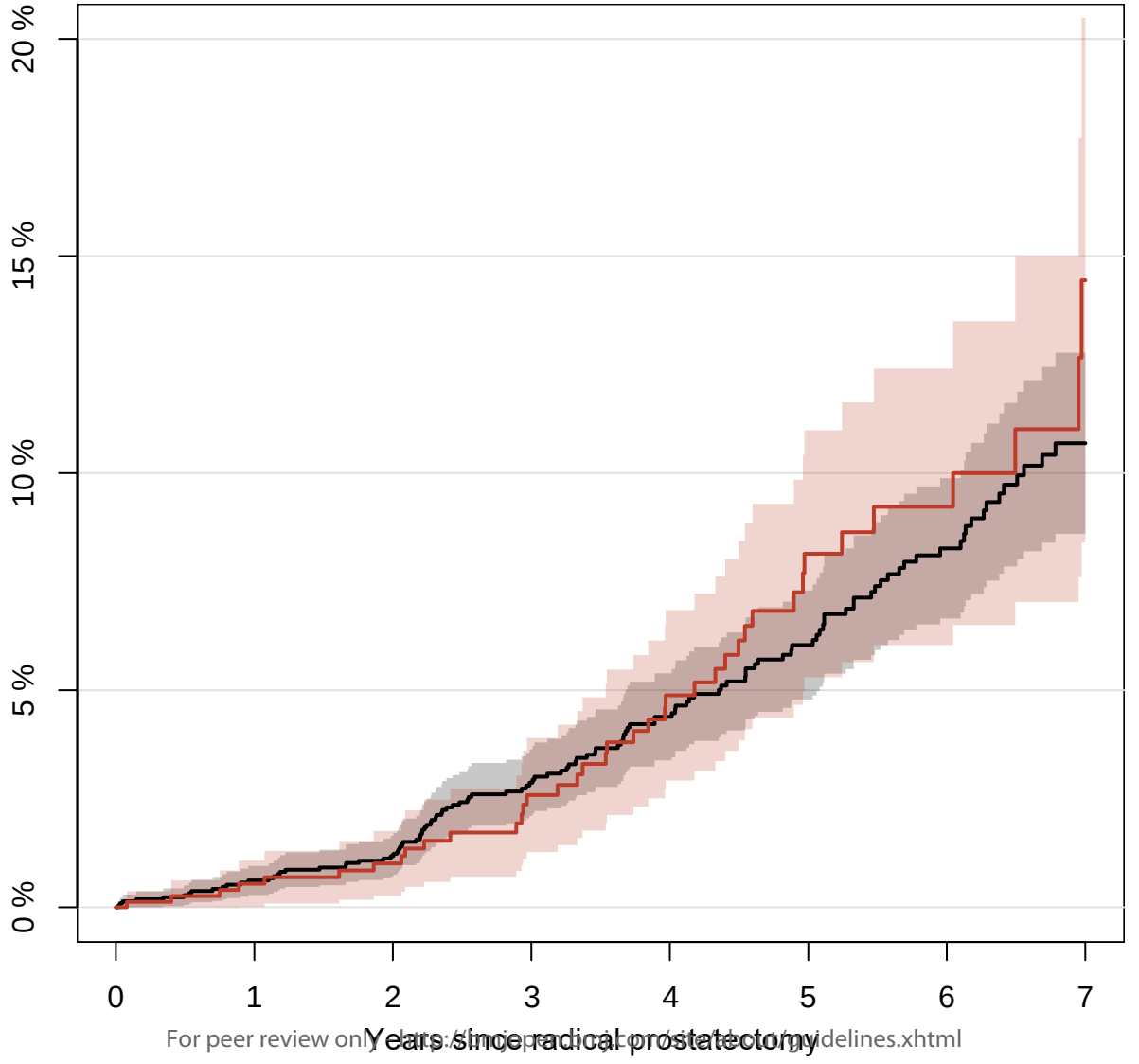


Figure 4. Unadjusted cumulative incidence of death
Unadjusted cumulative incidence of death with 95% confidence intervals for men with prostate cancer after undergoing radical prostatectomy, stratified by type of imaging modality. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.



APPENDIX 1

Table 1. Registry data used in the analysis.

Registry	Code
The Danish Cancer Registry	
ICD-10 diagnosis and morphologic codes	
Prostate cancer	DC61.9
Tumor stage	TNM
The Danish National Registry of Patients	
Imaging modality	
Danish Health Care Classification System, sks-codes	
Bone scintigraphy	WKBxx
^{18}F-NaF PET	WDTPSFCXX
The Danish National Registry of Patients	
Primary prostate cancer treatment	
NCSP codes	
Radical prostatectomy	KKECxx
The Danish National Registry of Patients	
Skeletal-related events	
NCSP codes	
Radiation to bone	BWGxx
Surgery to bone	KNAGxx
ICD-10 codes	
Bone metastases	DC79.5
Spinal cord compression	DG952
Pathological fractures	
Osteoporotic fractures	
The Danish Register of Laboratory Results	
NPU codes	
PSA	NPU0866
The Danish National Pathology Registry	
SNOMED codes	
Gleason score	ÆF0xx

NCSP: Nomesco Classification of Surgical Procedures; NPU: Nomenclature for Properties and Units; SNOMED: Systematized Nomenclature of Medicine

Table 2. Comorbidity codes from The Danish National Patient Registry used to calculate the Charlson Comorbidity Index. All codes are ICD-10 codes.

Comorbidity	Code
Myocardial infarction	DI21, DI22
Heart failure	DI099, DI110, DI130, DI132, DI255, DI425, DI426, DI427, DI429, DI428A, DP290, DI43, DI50, DE105, DE115, DE125, DE135, DE145
Peripheral vascular disease	DI70, DI71, DI72, DI731, DI738, DI739, DI77, DI790, DI792, DK551, DK558, DK559, DZ958, DZ959
Cerebrovascular disease	DI60, DI61, DI62, DI63, DI64, DI65, DI66, DI67, DI68, DI69, DG45, DG46, DH340
Dementia	DF00, DF01, DF02, DF03, DG30, DF051, DG311
Chronic pulmonary disease	DJ40, DJ41, DJ42, DJ43, DJ44, DJ45, DJ46, DJ47, DJ60, DJ61, DJ62, DJ63, DJ64, DJ65, DJ66, DJ67, DJ684, DI278, DI279, DJ84, DJ701, DJ703, DJ920, DJ953, DJ961, DJ982, DJ983
Rheumatic disease	DM05, DM06, DM08, DM09, DM30, DM31, DM32, DM33, DM34, DM35, DM35, DM36, D86
Peptic ulcer disease	DK25, DK26, DK27, DK28, DK221
Mild liver disease	DB18, DK700, DK701, DK702, DK709, DK703, DK713, DK714, DK715, DK717, DK73, DK74, DK760, DK762, DK763, DK764, DK769, DZ944
Severe liver disease	DB150, DB160, DB162, DB190, DI850, DI859, DI864, DI982, DK704, DK711, DK721, DK729, DK765, DK766, DK767
Diabetes without complications	DE100, DE101, DE108, DE109, DE110, DE111, DE119, DE120, DE121, DE129, DE130, DE131, DE139, DE140, DE141, DE149
Diabetes with complications	DE102, DE103, DE104, DE105, DE106, DE107, DE112, DE113, DE114, DE115, DE116, DE117, DE118, DE122, DE123, DE124, DE125, DE126, DE127, DE128, DE132, DE133, DE134, DE135, DE136, DE137, DE138, DE142, DE143, DE144, DE145, DE146, DE147, DE148
Hemiplegia paraplegia	DG830, DG831, DG832, DG833, DG834, DG81, DG82, DG041, DG114, DG801, DG802, DG839
Renal disease	DN032, DN033, DN034, DN035, DN036, DN037, DN052, DN053, DN054, DN055, DN056, DN057, DZ490, DZ491, DZ492, DN18, DN19, DI120, DI131, DI132, DN250, DZ940, DZ992, DN26
Any malignancy	DC0, DC1, DC2, DC3, DC40, DC41, DC42, DC43, DC44, DC45, DC46, DC47, DC48, DC49, DC5, DC6, DC70, DC71, DC72, DC73, DC74, DC75, DC76, DC86, DC97
Metastatic solidtumor	DC77, DC78, DC79, DC80
AIDS/HIV	DB20, DB21, DB22, DB23, DB24
Leukemia	DC91, DC92, DC93, DC94, DC95
Lymphoma	DC81, DC82, DC83, DC84, DC85, DC88, DC90, DC96

Figure 1. Unadjusted cumulative incidence with 95% confidence interval of skeletal-related events (SRE) in men after undergoing radical prostatectomy, restricted to men from the Capitol region of Denmark. Death was treated as a competing event. Red curve represents ^{18}F-NaF PET, black curve bone scintigraphy.

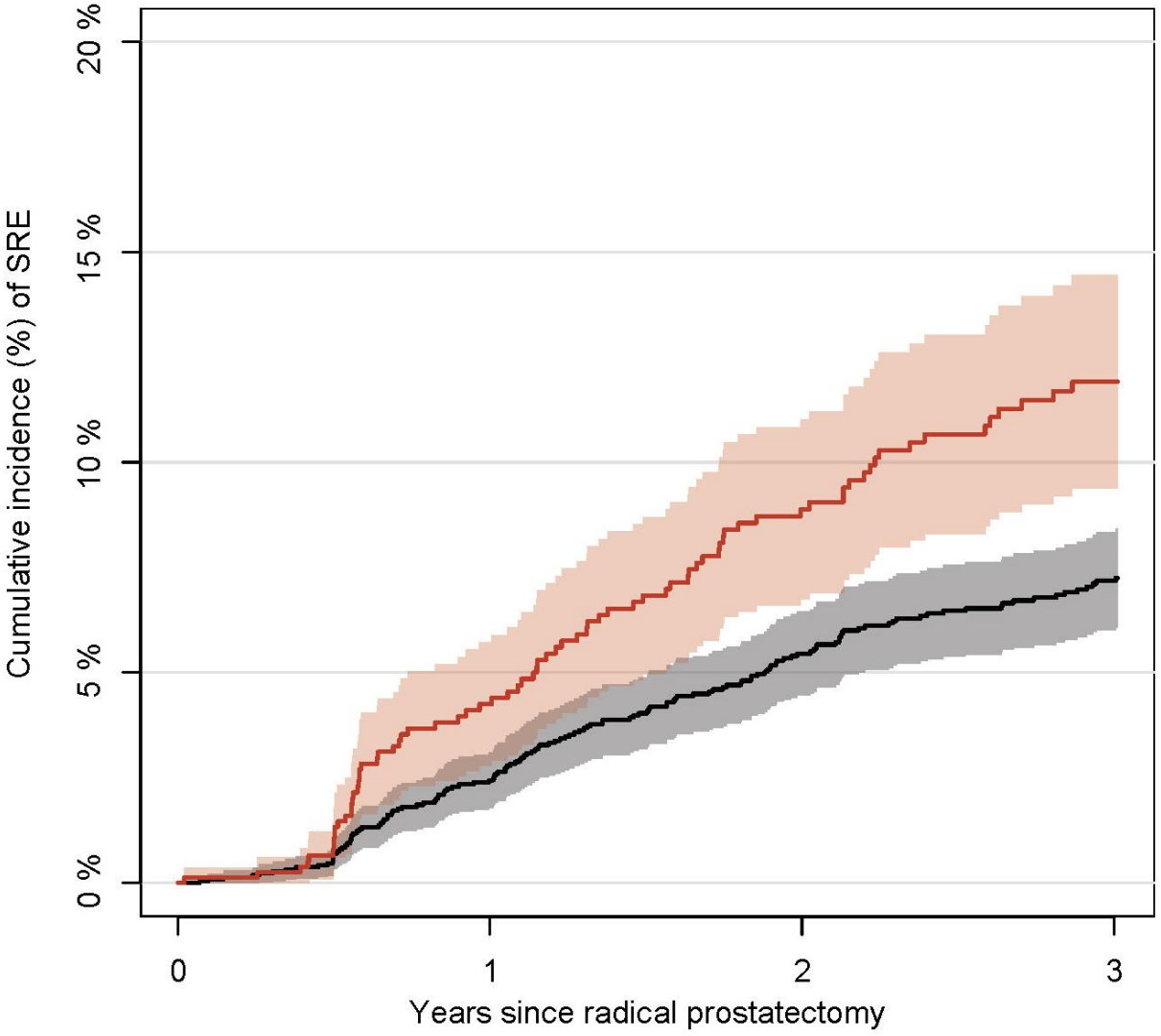


Figure 2. Hazard ratios for skeletal-related events following radical prostatectomy among patients receiving a ^{18}F-NaF PET before surgery compared with patients receiving a bone scintigraphy. Restricted to the Capitol region of Denmark.

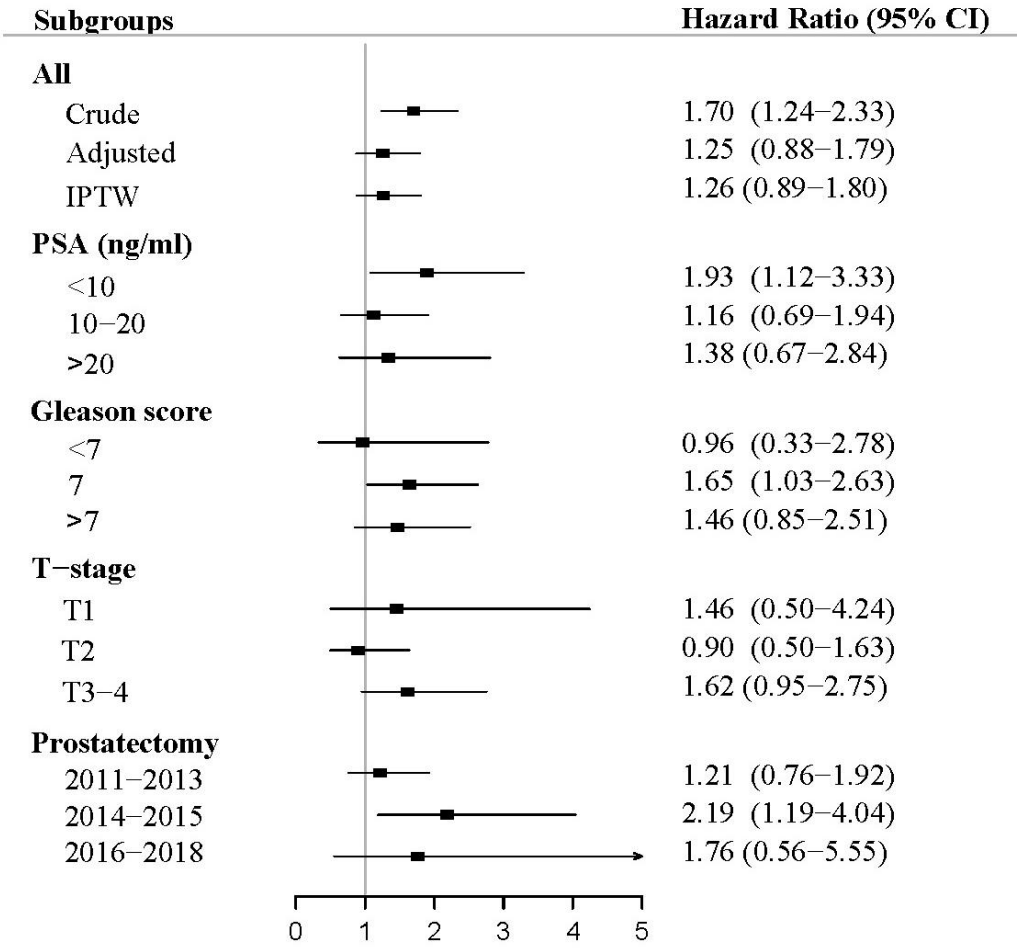


Table 3. Demographics for the Capitol region of Denmark. Baseline characteristics on the day of surgery for men with high-risk prostate cancer from 2011-2018* Stratified by pre-surgery imaging.

	Bone scintigraphy (n=690)	**^{18}F-NaF PET (n=740)**	**All (n=1,430)**
Age (year, median (IQR))	66.2 (60.8, 69.3)	67.9 (62.9, 71.1)	66.9 (61.9, 70.1)
Year of surgery			
2011-2013	331 (48.0)	212 (28.6)	543 (38.0)
2014-2015	185 (26.8)	231 (31.2)	416 (29.1)
2016-2018	174 (25.2)	297 (40.1)	471 (32.9)
PSA (ng/mL)			
<10	300 (44.1)	250 (34.4)	550 (39.1)
10-20	229 (33.6)	271 (37.3)	500 (35.5)
>20	152 (22.3)	206 (28.3)	358 (25.4)
Gleason score			
<7	81 (12.0)	61 (8.3)	142 (10.1)
7	401 (59.6)	432 (58.9)	833 (59.2)
>7	191 (28.4)	240 (32.7)	431 (30.7)
Clinical T-stage			
T1	58 (9.0)	39 (6.3)	97 (7.7)
T2	435 (67.3)	220 (35.8)	655 (51.9)
T3-T4	153 (23.7)	356 (57.9)	509 (40.4)
Comorbidity			
Cardiovascular diseases	35 (5.1)	46 (6.2)	81 (5.7)
Other malignancies	29 (4.2)	59 (8.0)	88 (6.2)
Diabetes	21 (3.0)	44 (6.0)	65 (4.6)
Charlson comorbidity index			
1	84 (12.2)	103 (13.9)	187 (13.1)
>1	59 (8.6)	97 (13.1)	156 (10.9)

*Percentages may not sum to 100 due to rounding or missing data
IQR: Interquartile range; PSA: Prostate specific antigen; T-stage: Tumor stage.

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

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Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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USE OF 18F-NaF-PET IN THE STAGING OF SKELETAL METASTASES OF NEWLY DIAGNOSED, HIGH-RISK PROSTATE CANCER PATIENTS - A NATIONWIDE COHORT STUDY.

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Secondary Subject Heading:	Urology, Epidemiology, Oncology
Keywords:	NUCLEAR MEDICINE, ONCOLOGY, Epidemiology < ONCOLOGY, Urological tumours < UROLOGY

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TITLE PAGE

USE OF ^{18}F-NaF-PET IN THE STAGING OF SKELETAL METASTASES OF NEWLY DIAGNOSED, HIGH-RISK PROSTATE CANCER PATIENTS - A NATIONWIDE COHORT STUDY.

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USE OF ^{18}F-NaF-PET IN THE STAGING OF SKELETAL METASTASES OF NEWLY DIAGNOSED, HIGH-RISK PROSTATE CANCER PATIENTS - A NATIONWIDE COHORT STUDY.

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ABSTRACT

Objective To determine whether preoperative staging of high-risk prostate cancer with ^{18}F-NaF PET reduces the risk of skeletal metastases.

Design Nationwide, population-based cohort study using real-world data.

Setting The study used national health registries, including all sites in Denmark from 2011-2018.

Participants Newly diagnosed high-risk prostate cancer patients who underwent radical prostatectomy from 2011-2018. Patients were stratified into two groups according to the preoperative imaging modality of either ^{18}F-NaF PET or bone scintigraphy.

Main outcome measures The risk of skeletal-related events as a proxy for skeletal metastases following radical prostatectomy. The secondary endpoint was overall survival.

Results Between January 1, 2011, and December 31, 2018, 4,183 high-risk patients underwent radical prostatectomy. Of these patients, 807 (19.3%) underwent ^{18}F-NaF PET and 2,161 (51.7%) underwent bone scintigraphy. The remaining 30% were examined by a different imaging method or did not undergo imaging. Using the inverse probability of treatment weighting to control potential confounding, the hazard ratio of experiencing a skeletal-related event for patients in the ^{18}F-NaF PET group versus the bone scintigraphy group was 1.15 (95% CI 0.86-1.54). The 3-year survival rates were 97.4% (95% CI 96.1-98.7) and 97.1% (95% CI 96.4-97.9) for patients receiving ^{18}F-NaF PET and bone scintigraphy, respectively.

Conclusion High-risk prostate cancer patients undergoing preoperative staging with ^{18}F-NaF PET did not display a lower risk of developing skeletal-related events after prostatectomy compared to patients undergoing bone scintigraphy. The survival rates were similar between the two groups.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Registry data provides real-world data on the clinical impact of clinical practices
- This study identified a large cohort from all institutions in Denmark using high-quality registry data.
- The routinely collected health data are not specifically registered for the purposes of this research, resulting in a minor degree of missing data.
- Regression analysis weighted by the inverse probability of treatment ensured consideration of all measured confounders and addressed confounding by indication.

INTRODUCTION

Prostate cancer is one of the most common malignancies in the Western world, with over 1.4 million new cases reported in 2020.[1] Prostate cancer frequently metastasizes to the bone, which is associated with significant morbidity and mortality.[2 3] Accurate detection of bone metastases at primary staging is essential for decision-making regarding subsequent management. At the time of diagnosis, the risk of recurrence is determined based on the PSA level, Gleason score, and clinical tumour stage (T-stage).[4] Patients classified as unfavorable–intermediate risk or high risk will often receive preoperative staging by imaging. International urology and oncology guidelines recommend bone scintigraphy with 99mTechnetium-labeled phosphonate (99mTc) for the assessment of bone metastases at primary staging.[4 5]

However, several studies have shown that the bone-specific positron emission tomography (PET) tracer ^{18}F-sodium-fluoride (^{18}F-NaF) is superior to bone scintigraphy in terms of its diagnostic accuracy for detecting bone metastases including fewer equivocal findings.[6-8] In previous studies, the sensitivity of bone scintigraphy for the detection of bone metastases varied from 57% to 97%, and the specificity varied from 57 to 80%.[6-9] In contrast, the sensitivity of ^{18}F-NaF PET for the diagnosis of bone metastases has ranged from 81 to 100% in the majority of studies, with a specificity ranging from 71 to 100%.[6-8 10 11] With the purported lower accuracy of bone scintigraphy, the risk of misdiagnosing patients is high, possibly resulting in suboptimal treatment strategies. Among patients referred for suspected metastases, the use of ^{18}F-NaF PET instead of bone scintigraphy in patients with prostate cancer has been shown to affect the patient management strategy in 6-12% of cases.[12 13] However, no studies have documented that the subsequent change in patient management strategies induced by ^{18}F-NaF PET and its improved diagnostic accuracy confer any patient benefit in terms of mortality, morbidity and quality of life. Thus, we performed a cohort study with real-world data of men diagnosed with prostate cancer in Denmark who underwent either bone scintigraphy or ^{18}F-NaF PET as part of primary staging before curative intent prostatectomy to examine whether the type of preoperative imaging modality was associated with overall survival and skeletal-related events (SREs) after radical prostatectomy.

METHODS

Study Population and Data Sources

This nationwide register-based cohort study was conducted in Denmark, which has approximately 5.8 million residents. In Denmark, all residents are provided with free, tax-supported health care by the National Health Service. A unique 10-digit civil registration number is assigned to all residents at birth by the Central Office of Civil Registration. This number allows unambiguous linkage across all Danish population-based registries.[14] Reporting to the registries by clinicians is mandatory, which ensures high completeness of medical information. The applied data included nationwide information from the Danish Cancer Registry,[15] the Civil Registration System,[16] the Danish National Patient Registry,[17] the Register of Laboratory Results for Research,[18] the Danish Prostate Cancer Database,[19] the Danish National Pathology Register,[20] and the Register of Causes of Death.[21] Appendix 1 (p 1) provides a detailed description of the codes found in the registries for prostate cancer characteristics, treatment, outcomes, and covariates. Furthermore, the study is reported in accordance with STROBE guidelines, and a checklist is provided in the supplementary files.

Identifying Men with Prostate Cancer

No formal screening program for prostate cancer existed during the study period. Therefore, men were referred to the urology department upon suspicion of prostate cancer. We used the Danish National Patient Registry to identify a cohort consisting of men with a first-time prostate cancer diagnosis from 2011 through 2018 who had undergone radical prostatectomy. This registry was established in 1977 for hospitalized patients; outpatient visits at hospitals have been included since 1995.[17] The registry includes dates of admission and discharge, diagnosis (ICD-10 codes), surgical procedures, and treatment information. The validity of a prostate cancer diagnosis in this register has previously been evaluated and found to be high, with a positive predictive value of nearly 90%.[22]

Risk Classification

We restricted the cohort to patients we could classify as having a preoperative high risk of cancer recurrence according to the European Association of Urology (EAU) risk classification of prostate cancer. The EAU defines high-risk patients as those with a PSA of more than 20 ng/mL OR a Gleason score >7 OR a T-stage of T2c as the minimum.[4] PSA values were retrieved from the Danish Register of Laboratory Results, which includes laboratory data from four of the five regions of Denmark.[18] Data from the last region were obtained directly from the relevant regional database. The Gleason score was obtained from the Pathology Register, which contains information on all pathological examinations conducted in Denmark since 1997. T-stage was obtained from the Danish Cancer Registry, which has prospectively recorded all cancers diagnosed in Denmark since 1943, classified according to ICD-10, and ICD Oncology codes (ICD-0-3) for topography and morphology.[15] For all three variables, we included the latest recorded value within six months prior to surgery. If PSA, Gleason score, or T-stage were missing, we used the Danish Prostate Cancer Database to fill in the

missing variables. This register is a nationwide clinical cancer database established in 2010 that records data on all incident, historically verified prostate cancer cases.

Imaging Modality

We retrieved information on imaging modalities from the Danish National Patient Registry. We identified the preoperative use of bone scintigraphy and ^{18}F-NaF PET, recorded up to 6 months before surgery, combined with computer tomography (CT) or magnetic resonance imaging (MRI). Single-photon emission (SPECT)/CT was conducted according to institutional practices. Patients were categorized according to their preoperative imaging into two groups: those who underwent bone scintigraphy only (bone scintigraphy group) and those who underwent ^{18}F-NaF PET scan with or without bone scintigraphy (^{18}F-NaF PET group). In general, each site performed only one of the two scans; thus, physicians did not stratify patients according to a specific imaging modality. Patients with an ^{18}F-NaF PET scan performed as a part of a clinical research project were excluded from the cohort because the results of these scans were not made available to the referring physician.

SREs and Bone Metastases

We obtained information on SREs through the Danish National Patient Registry. SREs comprised the following events occurring after the date of radical prostatectomy: radiation to the bone defined as 1-4 treatments with external radiation therapy (standard practice in Denmark for the treatment of bone pain), pathological and osteoporotic fractures, spinal cord compression, surgery to the bone, or a first-time bone metastasis diagnosis code.

Mortality

Mortality and migration updates were obtained from the Civil Registration System, which is updated daily.[14] The register contains information on the vital status (dead or alive), date of death, and migration status of all Danish citizens.

Comorbidity

We used the Charlson comorbidity index to describe preexisting comorbidities in the prostate cancer cohort[23] (appendix 1 p 2). We calculated the index based on diagnoses recorded in the Danish National Patient Registry up to ten years before the date of surgery. For analysis, we categorized the index into 3 comorbidity levels, including 1) those without comorbidity, 2) those with a comorbidity index equal to 1, and 3) those with a comorbidity index above 1.

Statistical Analysis

Baseline characteristics are reported as frequencies with percentages and medians with interquartile ranges. We estimated the cumulative risk of SREs according to the type of imaging modality and plotted the cumulative risk as a function of time since radical prostatectomy; death was treated as a competing risk event. Patients contributed time at risk from the date of radical prostatectomy until the date of first-time registered

SRE, migration, death, or December 31, 2018, whichever came first. Finally, we similarly estimated the cumulative incidence of death.

For the main analysis, we used Cox proportional hazards regression analysis to estimate the age-adjusted and multivariate-adjusted hazard ratios (HRs) of SREs with 95% CIs, comparing those who underwent ^{18}F-NaF PET scans with those who underwent bone scintigraphy. Additionally, to better control potential confounding by indication, analysis of the inverse probability of treatment weighting (IPTW) was performed based on the propensity score for ^{18}F-NaF PET. Propensity scores were calculated using logistic regression with the inclusion of the same variables as in the adjusted Cox analysis. We adjusted for age, Charlson comorbidity index, PSA (categorical variable: <10, 10-20, >20 ng/mL), Gleason score (categorical variable: <7, 7, >7), and T-stage (categorical variable: T1, T2, T3+T4). Adjusting with categorical variables was deemed necessary due to outliers and the limited number of records available on the outer areas of the scales. Furthermore, we stratified the analysis by PSA, Gleason score, T-stage, and year of radical prostatectomy. In the stratified analysis, we only adjusted for age and Charlson comorbidity index. An adjusted HR of death was also calculated. No further analyses were performed for patients with other types of imaging or no imaging before surgery.

Several sensitivity analyses were performed to test the robustness of our findings. First, due to potential site-related differences in risk factors among the included patients, we conducted an analysis restricted to the capitol region of Denmark, which performed most of the ^{18}F-NaF PET scans. Second, we executed the analysis with a reclassification of the exposure group to include patients with both scans. To account for missing data and enable adjustment for PSA, Gleason score, and T-stage we used multiple imputation using splines[24] with all the main analysis variables and the outcome variable in the model. We produced and combined 200 sets of imputations.

Statistical software

Data management and analyses were conducted in R 4.0.3 using RStudio 2020 (RStudio, PBC, Boston, MA) with the following packages: heaven, data.table, Publish, survival, stringr, mitools, smcfcs and ipw.

Ethics Approval

The Danish Data Protection Agency approved the use of data for this study (reference number 2008-58-0028). Furthermore, the study was granted approval by the Danish Patient Safety Authority to collect laboratory data (reference numbers 3-3013-3183/1 and 31-1522-37). Ethics approval is not required for historical register-based studies in Denmark.

Patient and public involvement

This study was observational and based on data from routine healthcare records. No patients were directly involved in the study.

RESULTS

Between January 1, 2011, and December 31, 2018, 36,910 men were diagnosed with prostate cancer in Denmark, of whom 8,726 (23.6%) underwent radical prostatectomy (Figure 1). Among those who underwent radical prostatectomy, 4,183 patients (47.9%) were classified as high risk according to the EAU preoperative staging criteria. A total of 2,161 (51.7%) high-risk patients undergoing surgery were evaluated for skeletal metastasis with bone scintigraphy only, and 807 (19.3%) men were evaluated with ^{18}F-NaF PET. Information on the PSA values, Gleason score, and T-stage from the registries ensured nearly 90% completeness of the high-risk classification, resulting in a large study population for our analysis. A notable proportion of high-risk patients (28.5%) underwent different imaging modalities or no imaging to evaluate bone metastasis, and a small portion of patients (0.5%) were excluded because they underwent project-related imaging. The median age at the date of radical prostatectomy was 67 years (interquartile range, 62-70.1), and the median follow-up from surgery was 4.1 years (interquartile range, 2.4-6.0 years). In general, patients receiving ^{18}F-NaF PET had a higher PSA level, Gleason score, and T-stage at primary staging (Table 1).

SREs and Bone Metastases

The unadjusted one-year cumulative risk of SREs was 2.4% (95% CI 1.8-3.1) for men who underwent bone scintigraphy and 4.3% (95% CI 2.8-5.7) for those who underwent ^{18}F-NaF PET (Figure 2). The unadjusted 3-year cumulative risk of SREs was 7.2% (95% CI 6.0-8.3) for men undergoing bone scintigraphy and 11.9% (95% CI 9.4-14.4) for those undergoing ^{18}F-NaF PET. Of the 300 men with at least one SRE recorded during follow-up, 53.7% had radiation to bone recorded as their first event, 30.7% had a pathological or osteoporotic fracture, 6.3% had spinal cord compression, 6.3% had a code for bone metastases, and 3.0% had bone surgery. In the main analysis, we did not find that ^{18}F-NaF PET decreased the HR of experiencing SREs after surgery; in contrast, we observed a slightly increased HR, which was reduced when adjusting the model (adjusted HR, 1.22; 95% CI 0.93-1.61; Figure 3). When we used IPTW to control for potential confounding factors, the risk of experiencing an SRE was attenuated (IPTW adjusted HR, 1.15: 95% CI 0.86-1.54; Figure 3). Stratified analyses similarly demonstrated increased HRs for SREs in patients undergoing ^{18}F-NaF PET compared to those undergoing bone scintigraphy, except for patients with stage 2 disease and those with a Gleason score <7 (Figure 3).

Survival

Figure 4 shows the cumulative survival curves of the cohorts for up to 7 years of follow-up. The one-year survival was 99.4% (95% CI 99.0-99.7) in men who underwent bone scintigraphy and 99.5% (95% CI 98.9-100) in men who underwent ^{18}F-NaF PET, and the corresponding 3-year survival rates in the cohorts were 97.1% (95% CI 96.4-97.9) and 97.4% (95% CI 96.1-98.7), respectively. Adjusted analyses showed a modest reduction in mortality for patients who underwent ^{18}F-NaF PET (adjusted HR, 0.89; 95% CI 0.61-1.30).

Sensitivity Analysis

Restricting to patients from the capitol region yielded cumulative SRE risk estimates consistent with those of the main analysis (appendix 1 p 3). Similar to the main analysis, the cumulative risk of SREs was higher for men evaluated with ^{18}F-NaF PET than for those evaluated with bone scintigraphy. Adjusted analysis for the capitol region was also comparable to the main analysis (appendix 1 p 4) and did not suggest any added value of using ^{18}F-NaF PET.

Including patients with both bone scintigraphy and ^{18}F-NaF PET in the bone scintigraphy group or excluding them entirely yielded HRs similar to those of the main analysis. A final analysis with imputed values for PSA, Gleason score, and T-stage yielded HRs similar to those of the analysis without imputation.

DISCUSSION

Principal findings

In this nationwide cohort study of Danish patients with high-risk prostate cancer undergoing prostatectomy, we found that primary staging with ^{18}F-NaF PET did not reduce the risk of SREs compared to primary staging with bone scintigraphy, whereas a slight tendency towards a reduction in all-cause mortality was observed in the group undergoing ^{18}F-NaF PET. To the best of our knowledge, this is the first study to evaluate patient-relevant outcomes of using a PET-based method for primary staging.

Comparison with other studies

Prior studies on ^{18}F-NaF PET in prostate cancer have focused on its improvements in diagnostic accuracy compared to bone scintigraphy[6-8] or its impact on patient management.[12 13] The superior diagnostic performance of ^{18}F-NaF PET when detecting bone metastases, should presumably result in improved patient selection for curative and life-prolonging treatment, resulting in fewer SREs the first few years after surgery. However, in this study, we did not observe any superiority over bone scintigraphy in terms of patient benefit among newly diagnosed, high-risk prostate cancer patients.

Evidence of patient-relevant outcomes is often reported from randomized controlled trials. Randomized trials are, however, not commonly conducted within the field of imaging, and it has previously been debated whether randomized trials are necessary to evaluate diagnostic procedures.[25 26] In prostate cancer, only two randomized controlled trials have been published, employing PET in one arm and standard imaging in the other arm. One such trial confirmed the diagnostic superiority of PSMA PET/CT during primary staging[27], whereas the other trial focused on the changes in patient management based on fluciclovine PET/CT at the time of biochemical recurrence;[28] none of these trials were linked to patient-relevant outcomes.

Randomized trials have demonstrated the clinical benefit of PET within other types of cancers, such as haematological and lung cancers.[29] Fischer et al. compared preoperative staging with FDG PET/CT to conventional staging by CT in lung cancer patients and found that patients in the PET/CT group showed a reduction in both the total number of thoracotomies and the number of futile thoracotomies; however, they did not observe a decrease in overall mortality.[30] Similar results were reported for colorectal liver metastases, with one study finding that FDG PET led to a reduction in futile laparotomies in 1 of 6 patients.[31] It could be expected that the use of ^{18}F-NaF PET would reduce the number of "futile" prostatectomies in patients harbouring bone metastases at the time of diagnosis, thereby reducing the incidence of SREs postoperatively. With recent trials demonstrating superior diagnostic properties of PSMA PET for primary staging in high-risk prostate cancer, its impact on treatment choice—and perhaps outcome—is likely to be greater than that of ^{18}F-NaF PET.

Strengths and limitations

The major strengths of our study are its national scale, large cohort, high-quality registry data, and complete follow-up. The registration of information related to prostate cancer diagnosis and radical prostatectomy, as well as variables defining the high-risk population, is thought to be practically complete because of a uniformly organized health care system where healthcare is free (tax-supported) and available to all residents.[32] Furthermore, a median follow-up time of 4.1 years is adequate for the purpose of evaluating bone metastases not captured by the imaging modality at primary staging; hence, only patients with a negative scan will undergo radical prostatectomy with curative intend in Denmark.

Nevertheless, our study has several limitations worth considering. The potential of confounding by indication was particularly concerning because of the observed higher values for PSA, Gleason score, and T-stage in the ^{18}F-NaF PET group; however, the indication of usage was the same for both scans. Moreover, the demographics of the groups might have been more alike if the International Society of Urological Pathology (ISUP) grading system was used for the Gleason score, which distinguishes between normal high-risk prostate cancer and very high-risk (ISUP grade 5) cancer cases. It was not possible to use the ISUP grading due to unavailability in some of the registers. Furthermore, confounding by indication is only an issue in hospitals that offer both bone scintigraphy and ^{18}F-NaF PET, which is highly uncommon in Denmark. Since sites only used one of the imaging modalities, physicians did not have to choose between the two, resulting in minimal selection bias. We attempted to control for confounding by using a propensity score-based inverse probability of treatment weighting, but we cannot rule out residual confounding due to misclassified or unmeasured prognostic factors. Multi-parametric magnetic resonance imaging (mpMRI) is also a factor worth considering in relation to targeted biopsies in the diagnostic work-up of prostate cancer. This method has been gradually implemented nationally in Denmark and prior to 2018 only very few sites had access to mpMRI for all patients, hence; we do not have data available yet. The introduction of mpMRI targeted biopsy is likely to affect the selection of patients for RP in the future

In the present study, we defined SREs as either external radiation therapy, pathological or osteoporotic fractures, spinal cord compression, surgery to the bone, or a bone metastases code. It can be speculated that patients treated at a site using ^{18}F-NaF PET would undergo ^{18}F-NaF PET rather than bone scintigraphy in case of biochemical recurrence, thereby increasing the detection of bone metastases during follow-up. However, the risk of SREs was primarily driven by a high percentage of radiotherapy of bone or fracture cases, which are not related to ^{18}F-NaF PET. Moreover, with the widespread introduction of prostate specific membrane antigen (PSMA) PET/CT in Denmark from 2015 and onwards, patients with biochemical recurrence would undergo PSMA PET/CT rather than ^{18}F-NaF PET/CT. Information regarding bone metastases was noted in only 6.3% of SREs across the groups.

Conclusions

In conclusion, we found that the use of ^{18}F-NaF PET at primary staging did not improve patient-relevant outcomes in terms of a reduction in SREs compared to that with bone scintigraphy.

ACKNOWLEDGMENT SECTION

Author Contributions

AWM, LJP and HDZ conceived the study and contributed to the literature search. AWM and CTP had access to the data and carried out the data management and analysis. AWM, CTP and MN designed the graphs. AWM, LJP, HDZ, CTP, MN and MTP aided in the interpretation of results. AWM prepared the first draft of the paper. All authors contributed to critical revision and approval of the final draft of the paper.

Conflict of Interest Disclosure

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/ coi_disclosure.pdf and declare: one author received grants from Bayer and Novo Nordisk for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Data availability statement

Data is not available to other researchers due to Registry or institutional database of patients providing routinely collected data.

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FIGURE LEGENDS

(Figure 1-4 are attached as separate PDF files)

Figure 1: Study profile

Study cohort of 2,161 men undergoing presurgical imaging with bone scintigraphy and 807 men undergoing ^{18}F-NaF PET. Patients with no or other imaging were combined since there were no differences between sites performing ^{18}F-NaF PET or bone scintigraphy. Moreover, we experienced inconsistencies in the way CT and MR scans where coded in the registries, making it difficult to distinguish between imaging of the prostate and other sites.

Figure 2: Unadjusted cumulative incidence of skeletal-related events (SREs)

The unadjusted cumulative incidence with 95% confidence intervals of SREs in men after undergoing radical prostatectomy. Death was treated as a competing event. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

Figure 3: Main analysis results

Hazard ratios for SREs following radical prostatectomy among patients undergoing 18F-NaF PET before surgery versus patients undergoing bone scintigraphy.

Figure 4: Unadjusted cumulative incidence of death

Unadjusted cumulative incidence of death with 95% confidence intervals for men with prostate cancer after undergoing radical prostatectomy, stratified by type of imaging modality. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

Table 1: Baseline patient characteristics by imaging modality

Characteristics on the day of surgery for men with high-risk prostate cancer from 2011 to 2018. Percentages may not add up to 100 due to rounding or missing data. *Top 3 comorbidities. Abbreviations: IQR, interquartile range; PSA, prostate specific antigen; T-stage, tumour stage.

TABLES

Table 1. Baseline patient characteristics by imaging modality

	Bone scintigraphy (n = 2,161)	^{18}F-NaF PET (n = 807)	All (n = 2,968)
Age (years, median (IQR))	66.3 (61.7, 69.7)	67.9 (62.9, 71.2)	66.7 (62.0, 70.1)
Year of surgery			
2011-2013	852 (39.4)	212 (26.3)	1,064 (35.8)
2014-2015	602 (27.9)	235 (29.1)	837 (28.2)
2016-2018	707 (32.7)	360 (44.6)	1,067 (36.0)
Imaging date before prostatectomy (days, median (IQR))	46 (32, 65)	42 (28, 56)	45 (30, 63)
PSA (ng/mL)			
<10	955 (45.0)	263 (33.1)	1,218 (41.8)
10-20	642 (30.2)	292 (36.8)	934 (32.0)
>20	526 (24.8)	239 (30.1)	765 (26.2)
Gleason biopsy score			
<7	345 (16.2)	70 (8.8)	415 (14.2)
7	1225 (57.5)	469 (58.6)	1,694 (57.8)
>7	560 (26.3)	261 (32.6)	821 (28.0)
Clinical T-stage			
T1	259 (12.6)	50 (7.5)	309 (11.4)
T2	1260 (61.5)	241 (36.0)	1,501 (55.2)
T3-T4	529 (25.8)	378 (56.5)	907 (33.4)
Comorbidity*			
Cardiovascular diseases	118 (5.5)	52 (6.4)	170 (5.8)
Other malignancies	102 (4.7)	64 (7.9)	166 (5.6)
Diabetes	62 (2.9)	48 (6.0)	110 (3.7)
Charlson comorbidity index			
1	267 (12.4)	115 (14.3)	382 (12.9)
>1	203 (9.4)	107 (13.3)	310 (10.4)

Characteristics on the day of surgery for men with high-risk prostate cancer from 2011 to 2018. Percentages may not add up to 100 due to rounding or missing data. *Top 3 comorbidities. Abbreviations: IQR, interquartile range; PSA, prostate specific antigen; T-stage, tumour stage.

Figure 1: Study profile

Study cohort of 2,161 men undergoing presurgical imaging with bone scintigraphy and 807 men undergoing 18F-NaF PET.

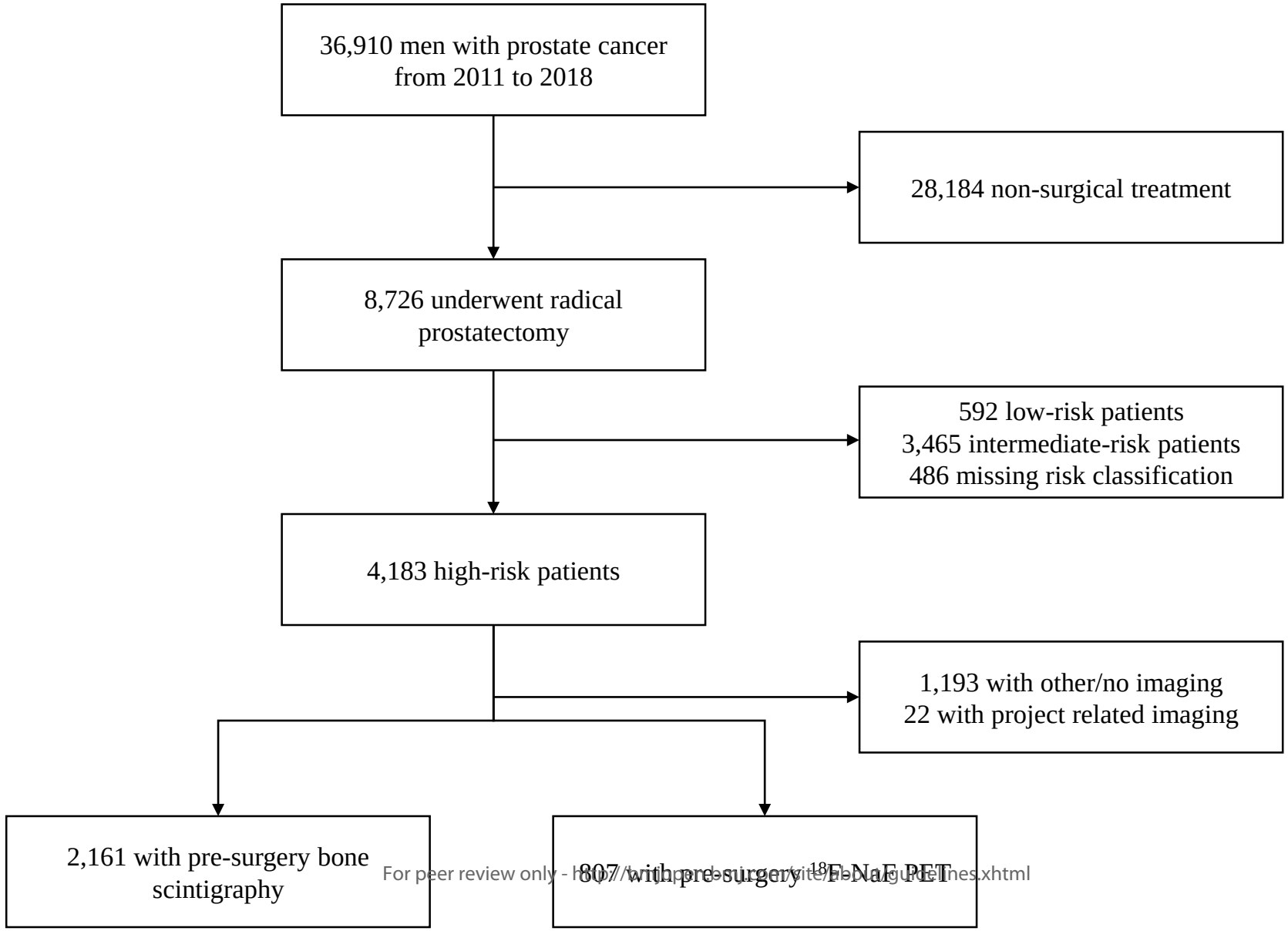


Figure 2. Unadjusted cumulative incidence of skeletal-related events (SREs)

The unadjusted cumulative incidence with 95% confidence intervals of SREs in men after undergoing radical prostatectomy. Death was treated as a competing event. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.

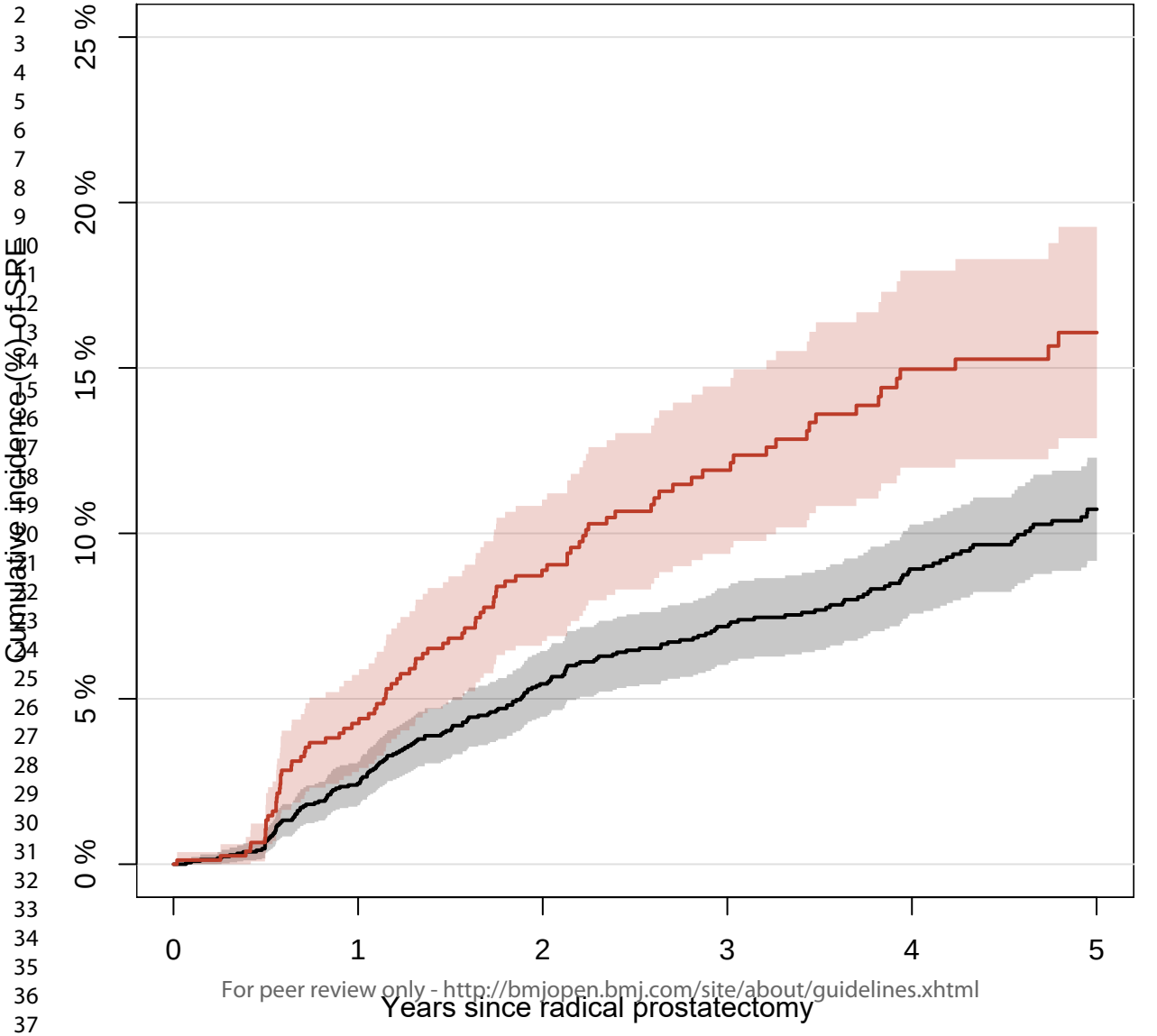


Figure 3. Main analysis results

Hazard ratios for SREs following radical prostatectomy among patients undergoing 18F-NaF PET before surgery versus patients undergoing bone scintigraphy.

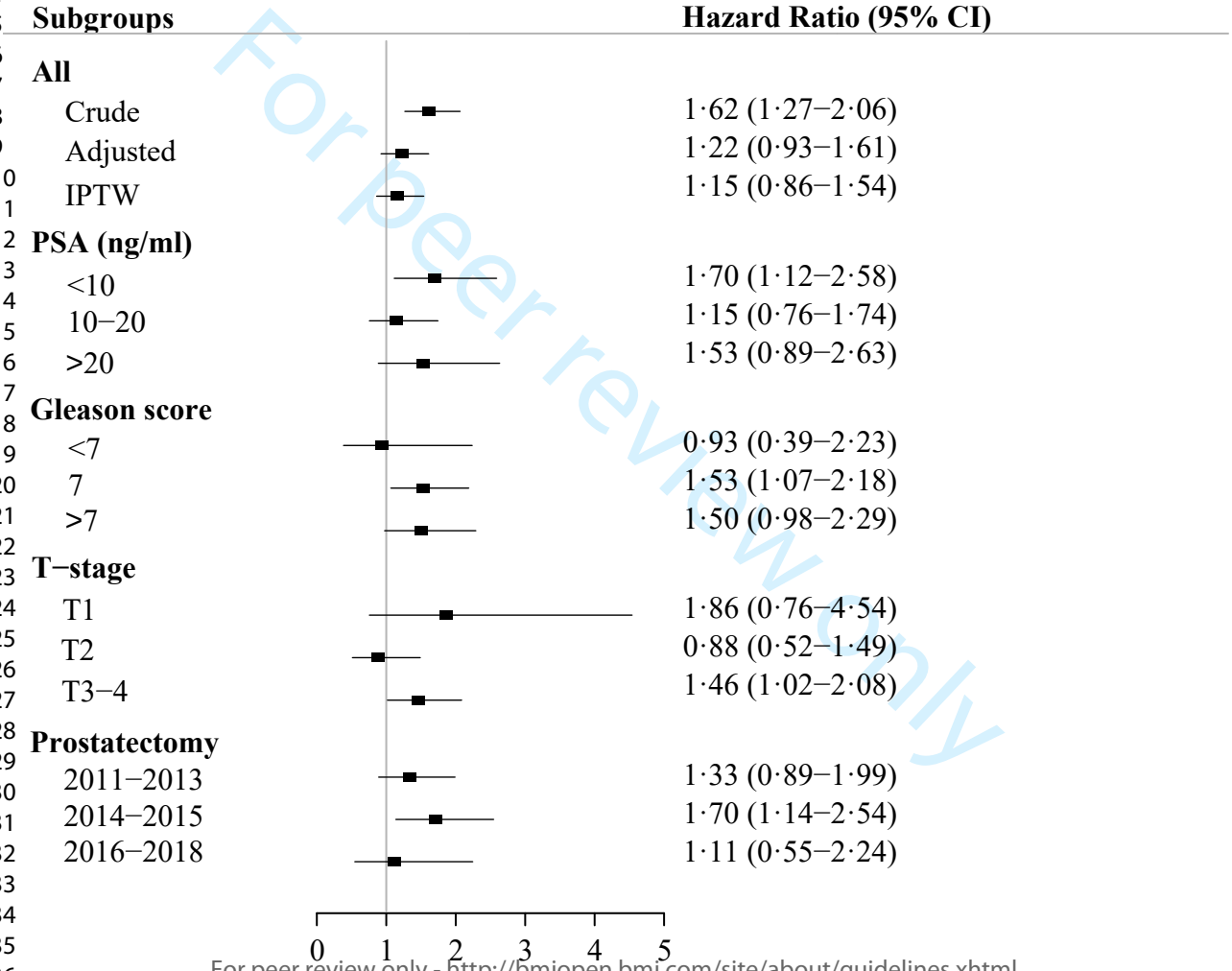
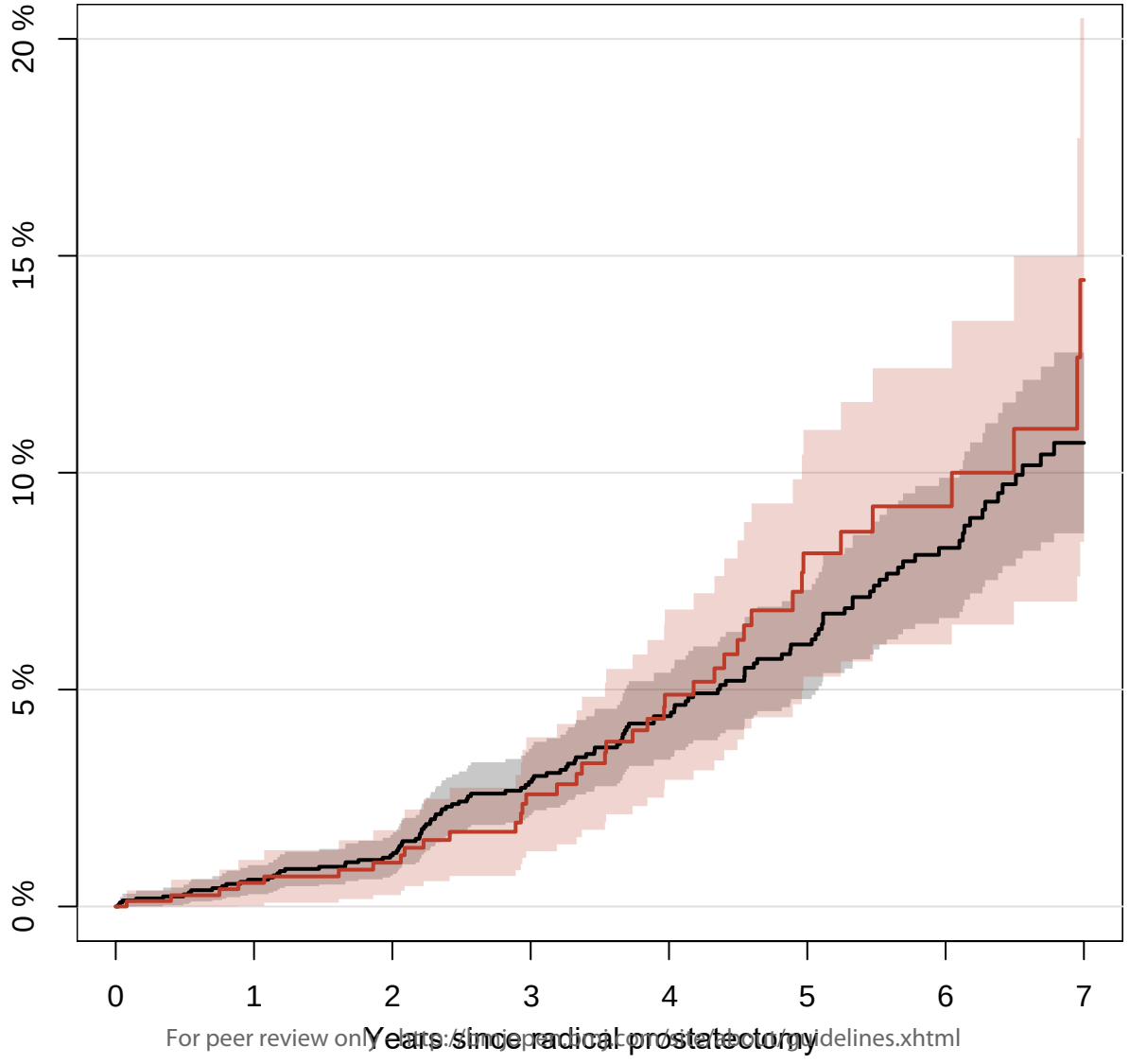


Figure 4. Unadjusted cumulative incidence of death

Unadjusted cumulative incidence of death with 95% confidence intervals for men with prostate cancer after undergoing radical prostatectomy, stratified by type of imaging modality. The red curve represents 18F-NaF PET, and the black curve represents bone scintigraphy.



APPENDIX 1

Table 1. Registry data used in the analysis.

Registry	Code
The Danish Cancer Registry	
ICD-10 diagnosis and morphologic codes	
Prostate cancer	DC61.9
Tumor stage	TNM
The Danish National Registry of Patients	
Imaging modality	
Danish Health Care Classification System, sks-codes	
Bone scintigraphy	WKBxx
^{18}F-NaF PET	WDTPSFCXX
The Danish National Registry of Patients	
Primary prostate cancer treatment	
NCSP codes	
Radical prostatectomy	KKECxx
The Danish National Registry of Patients	
Skeletal-related events	
NCSP codes	
Radiation to bone	BWGxx
Surgery to bone	KNAGxx
ICD-10 codes	
Bone metastases	DC79.5
Spinal cord compression	DG952
Pathological fractures	
Osteoporotic fractures	
The Danish Register of Laboratory Results	
NPU codes	
PSA	NPU0866
The Danish National Pathology Registry	
SNOMED codes	
Gleason score	ÆF0xx

NCSP: Nomesco Classification of Surgical Procedures; NPU: Nomenclature for Properties and Units; SNOMED: Systematized Nomenclature of Medicine

Table 2. Comorbidity codes from The Danish National Patient Registry used to calculate the Charlson Comorbidity Index. All codes are ICD-10 codes.

Comorbidity	Code
Myocardial infarction	DI21, DI22
Heart failure	DI099, DI110, DI130, DI132, DI255, DI425, DI426, DI427, DI429, DI428A, DP290, DI43, DI50, DE105, DE115, DE125, DE135, DE145
Peripheral vascular disease	DI70, DI71, DI72, DI731, DI738, DI739, DI77, DI790, DI792, DK551, DK558, DK559, DZ958, DZ959
Cerebrovascular disease	DI60, DI61, DI62, DI63, DI64, DI65, DI66, DI67, DI68, DI69, DG45, DG46, DH340
Dementia	DF00, DF01, DF02, DF03, DG30, DF051, DG311
Chronic pulmonary disease	DJ40, DJ41, DJ42, DJ43, DJ44, DJ45, DJ46, DJ47, DJ60, DJ61, DJ62, DJ63, DJ64, DJ65, DJ66, DJ67, DJ684, DI278, DI279, DJ84, DJ701, DJ703, DJ920, DJ953, DJ961, DJ982, DJ983
Rheumatic disease	DM05, DM06, DM08, DM09, DM30, DM31, DM32, DM33, DM34, DM35, DM35, DM36, D86
Peptic ulcer disease	DK25, DK26, DK27, DK28, DK221
Mild liver disease	DB18, DK700, DK701, DK702, DK709, DK703, DK713, DK714, DK715, DK717, DK73, DK74, DK760, DK762, DK763, DK764, DK769, DZ944
Severe liver disease	DB150, DB160, DB162, DB190, DI850, DI859, DI864, DI982, DK704, DK711, DK721, DK729, DK765, DK766, DK767
Diabetes without complications	DE100, DE101, DE108, DE109, DE110, DE111, DE119, DE120, DE121, DE129, DE130, DE131, DE139, DE140, DE141, DE149
Diabetes with complications	DE102, DE103, DE104, DE105, DE106, DE107, DE112, DE113, DE114, DE115, DE116, DE117, DE118, DE122, DE123, DE124, DE125, DE126, DE127, DE128, DE132, DE133, DE134, DE135, DE136, DE137, DE138, DE142, DE143, DE144, DE145, DE146, DE147, DE148
Hemiplegia paraplegia	DG830, DG831, DG832, DG833, DG834, DG81, DG82, DG041, DG114, DG801, DG802, DG839
Renal disease	DN032, DN033, DN034, DN035, DN036, DN037, DN052, DN053, DN054, DN055, DN056, DN057, DZ490, DZ491, DZ492, DN18, DN19, DI120, DI131, DI132, DN250, DZ940, DZ992, DN26
Any malignancy	DC0, DC1, DC2, DC3, DC40, DC41, DC42, DC43, DC44, DC45, DC46, DC47, DC48, DC49, DC5, DC6, DC70, DC71, DC72, DC73, DC74, DC75, DC76, DC86, DC97
Metastatic solidtumor	DC77, DC78, DC79, DC80
AIDS/HIV	DB20, DB21, DB22, DB23, DB24
Leukemia	DC91, DC92, DC93, DC94, DC95
Lymphoma	DC81, DC82, DC83, DC84, DC85, DC88, DC90, DC96

Figure 1. Unadjusted cumulative incidence with 95% confidence interval of skeletal-related events (SRE) in men after undergoing radical prostatectomy, restricted to men from the Capitol region of Denmark. Death was treated as a competing event. Red curve represents ^{18}F-NaF PET, black curve bone scintigraphy.

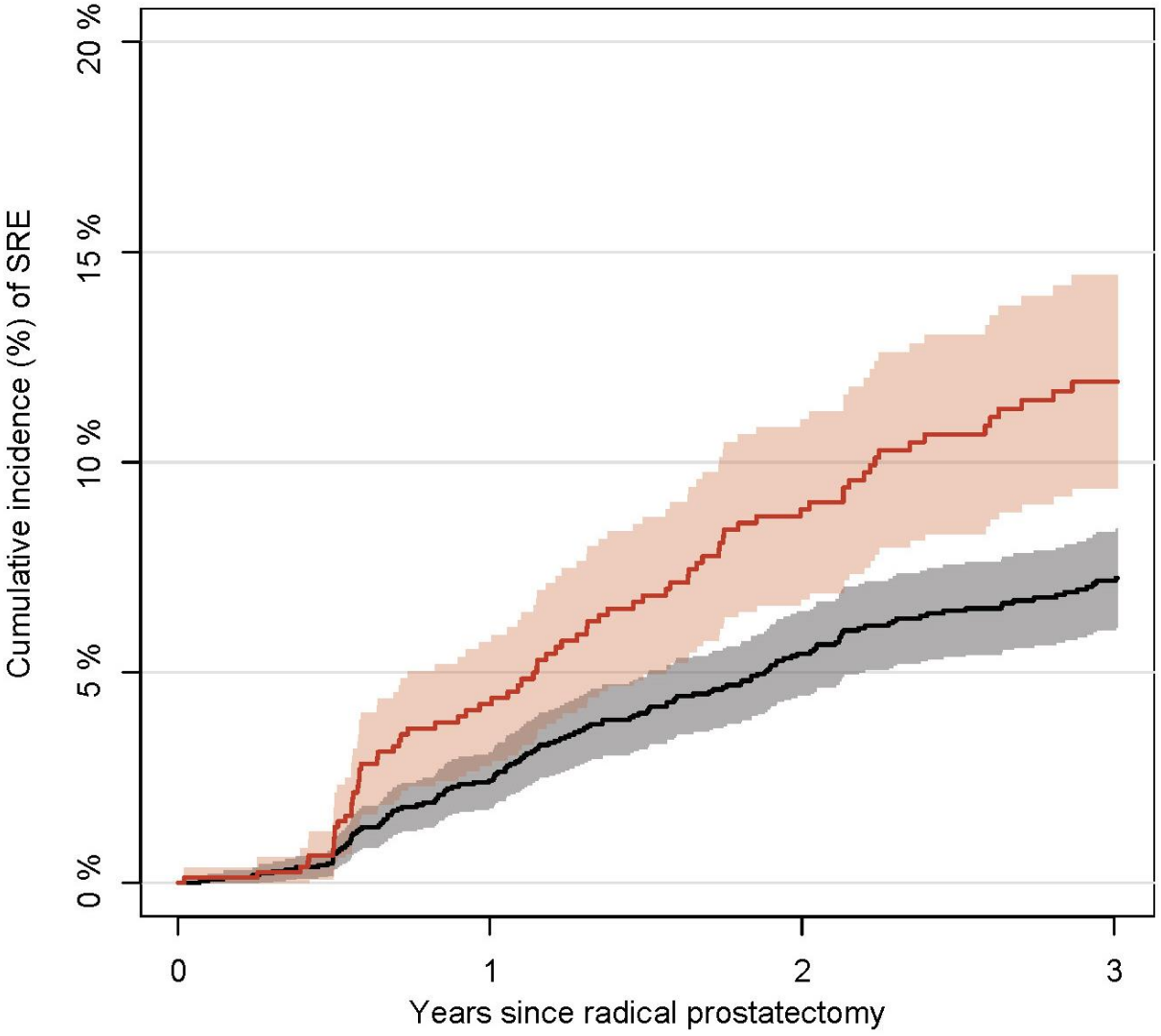


Figure 2. Hazard ratios for skeletal-related events following radical prostatectomy among patients receiving a ^{18}F-NaF PET before surgery compared with patients receiving a bone scintigraphy. Restricted to the Capitol region of Denmark.

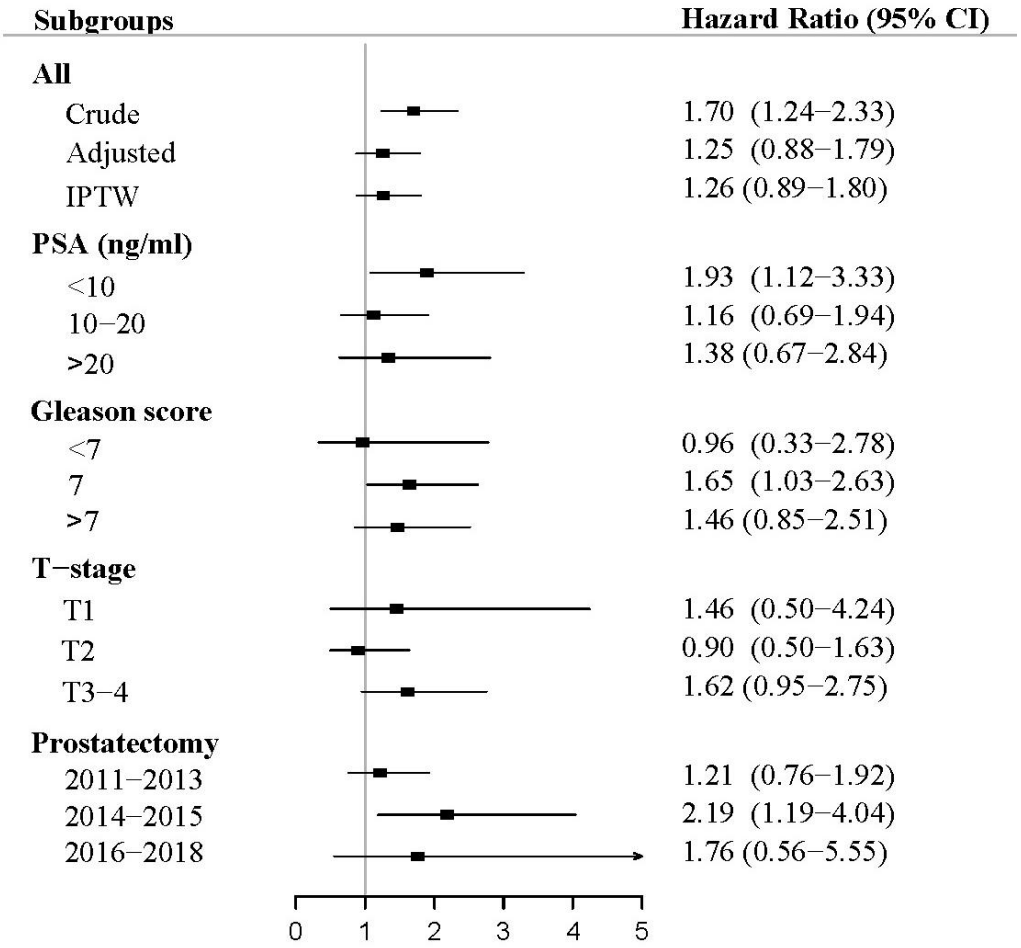


Table 3. Demographics for the Capitol region of Denmark. Baseline characteristics on the day of surgery for men with high-risk prostate cancer from 2011-2018* Stratified by pre-surgery imaging.

	Bone scintigraphy (n=690)	**^{18}F-NaF PET (n=740)**	**All (n=1,430)**
Age (year, median (IQR))	66.2 (60.8, 69.3)	67.9 (62.9, 71.1)	66.9 (61.9, 70.1)
Year of surgery			
2011-2013	331 (48.0)	212 (28.6)	543 (38.0)
2014-2015	185 (26.8)	231 (31.2)	416 (29.1)
2016-2018	174 (25.2)	297 (40.1)	471 (32.9)
PSA (ng/mL)			
<10	300 (44.1)	250 (34.4)	550 (39.1)
10-20	229 (33.6)	271 (37.3)	500 (35.5)
>20	152 (22.3)	206 (28.3)	358 (25.4)
Gleason score			
<7	81 (12.0)	61 (8.3)	142 (10.1)
7	401 (59.6)	432 (58.9)	833 (59.2)
>7	191 (28.4)	240 (32.7)	431 (30.7)
Clinical T-stage			
T1	58 (9.0)	39 (6.3)	97 (7.7)
T2	435 (67.3)	220 (35.8)	655 (51.9)
T3-T4	153 (23.7)	356 (57.9)	509 (40.4)
Comorbidity			
Cardiovascular diseases	35 (5.1)	46 (6.2)	81 (5.7)
Other malignancies	29 (4.2)	59 (8.0)	88 (6.2)
Diabetes	21 (3.0)	44 (6.0)	65 (4.6)
Charlson comorbidity index			
1	84 (12.2)	103 (13.9)	187 (13.1)
>1	59 (8.6)	97 (13.1)	156 (10.9)

*Percentages may not sum to 100 due to rounding or missing data

IQR: Interquartile range; PSA: Prostate specific antigen; T-stage: Tumor stage.

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.