Radical prostatectomy for clinically localised prostate cancer at Rigshospitalet 1995-2011.

An analysis of surgical and oncological outcome.

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LIST OF ABBREVIATIONS:

PCa	Prostate cancer
RP	Radical prostatectomy
PSA	Prostate specific antigen
GS	Gleason score
BR	Biochemical recurrence
BRFS	Biochemical recurrence-free survival
PSM	Positive surgical margin
DVC	Dorsal venous complex
OR	Odds ratio
HR	Hazard ratio
PPB	Percent positive biopsies for cancer = number of positive biopsies/Total number of biopsies.
95%CI	95% confidence intervals

ARTICLES INCLUDED IN THE PHD-THESIS:

1. Røder MA, Berg KD, Gruschy L, Brasso K, Iversen P. First Danish Single-institution Experience with Radical Prostatectomy: Biochemical outcome in 1200 consecutive patients. Prostate Cancer. 2011;2011:236357. doi:10.1155/2011/236357. Epub 2010 Dec 22.
2. Røder MA, Berg KD, Christensen IJ, Gruschy L, Brasso K, Iversen P. Radical Prostatectomy in Clinically Localized High-risk Prostate Cancer: Outcome of 231 consecutive patients. Scand J Urol. 2013 Feb;47(1):19-25. Epub 2012 Jul 5.
3. Vrang ML, Røder MA, Vainer B, Christensen IJ, Gruschy L, Brasso K, Iversen P. First Danish Single-institution Experience with Radical Prostatectomy: Impact of Surgical Margins on Biochemical Outcome. Scand J Urol Nephrol. 2012 Jun;46(3):172-9. Epub 2012 Feb 9.
4. Røder MA, Kawa SM, Scheike, Toft BG, Hansen JB, Brasso K, Vainer B, Iversen P. Risk of Biochemical Recurrence in Apical and Non-Apical pT2 Positive Surgical Margins after Radical Prostatectomy for Clinically Localized Prostate Cancer. Submitted to BJU International 12/8/13.:
5. Røder MA, Thomsen FB, Christensen IJ, Toft BG, Brasso K, Vainer B, Iversen P. Risk factors associated with positive surgical margins following radical prostatectomy for clinically localized prostate cancer: Can nerve-sparing surgery increase the risk? Scand J Urol. 2012 Nov 27. [Epub ahead of print]
6. Røder MA, Thomsen FB; Berg KD, Christensen IJ, Brasso K, Vainer B, Iversen P. Risk of biochemical recurrence and positive surgical margins in patients with pT2 prostate cancer undergoing radical prostatectomy. Impact of nerve-sparing surgery. Accepted for publication in Journal of Surgical Oncology 27/09/13.

INTRODUCTION

RP for clinically localized PCa was performed for the first time in Denmark in August 1995 at Rigshospitalet, Copenhagen. Since then, the Danish PCa epidemiology changed dramatically with a sharp increase in incidence and an increment of newly diagnosed clinically localized PCa. Surgery for clinically localized PCa soon spread throughout Denmark and RP is today being performed at six different centres.

The purpose of this PhD-thesis is to describe the first Danish experience with RP through analysis of data from the large prospective cohort of PCa patients with clinically localized disease who underwent RP and were followed at Rigshospitalet between 1995 and 2011. The thesis focuses on surgical and oncological outcome following RP.

BACKGROUND

PROSTATE CANCER: AN OVERVIEW.

The prostate gland is part of the male reproductive system and develops under androgen stimulation that, via multiple metabolic actions, promotes growth and several biological functions. The prostate consists of an epithelium, stromal cells and tissue matrix that act together to produce secretory proteins that facilitate semen coagulation and liquefaction. PSA is one of the predominantly secreted proteins from the prostate gland [1]. During the 70'ies, PSA could be analysed in seminal plasma, prostatic tissue and finally in serum [2]. PSA proved to be a useful marker of early PCa diagnosis and monitoring of patients treated for PCa [3]. Already in the early 90'ies, PSA was used as a clinical marker of PCa although its clinical and laboratory limitations were also emphasised [4]. Another major contribution to early diagnosis of PCa was facilitated through the fast improvements in ultrasound technology. In the late 80'ies, trans rectal ultrasound with systematic guided biopsies of the prostate gland became clinical routine and has since remained the gold-standard for image-guided diagnosis and intervention (e.g. seed implantation) to the prostate [5].

Malignant tumours in the prostate almost always develops as an adenocarcinoma. The most widely used grading system for the glandular pattern of the tumour is the Gleason score developed by Donald Gleason and George Mellinger in 1974 [6]. The score proved to be related to prognosis of PCa in both treated and untreated patients [7, 8]. Originally the score was calculated as the sum of the primary predominant pattern and the secondary pattern (second most prevalent). In 2005, the International Society of Urological Pathology (ISUP) modified the Gleason system, which widened the scope of Gleason pattern 4 and narrowed the definition of Gleason pattern 3 [9]. Further, it was agreed to evaluate Gleason score on biopsies as the primary pattern plus the highest grade and to ignore any lower grade that consisted of less than 5% of the total tumour volume. This modification has changed the Gleason score landscape of newly diagnosed PCa with a significant increment in patients diagnosed with Gleason score 7. This effect of the new ISUP guidelines on biopsy Gleason score has also been demonstrated in Danish patients who undergo RP [10]. It has been speculated that PCa develops from precursor lesions known as high-grade prostatic intraepithelial neoplasia. Theoretically, the tissue then transforms to a localised non-palpable (T1), but biopsy-detectable, tumour and grows within the prostate to become palpable at digital rectal examination (T2). Local spread involves dissemination through extracapsular extension and seminal vesicle invasion (T3). At this point the risk of locoregional lymph node metastasis increases and ultimately the tumour spreads as distant metastasis, primarily to the bone. The natural history of this process has been investigated in observational studies with patients managed expectantly for localised PCa. These studies demonstrated that the process of progression from localised disease to death of PCa might take more than 20 and depend on clinical stage and Gleason score at diagnosis [7, 11]. Although it is evident from observational studies that many localised PCa tumours are biologically indolent, the concept of definitive therapy of PCa became increasingly popular during the 80'ies and 90'ies. Throughout the past two decades a number of treatments for localised PCa emerged as routine therapy, including radical prostatectomy, external beam radiation therapy, and brachytherapy. With the technological evolution experimental treatments such as high-intensity focused ultrasound and cryotherapy of the prostate have been proposed as new therapeutic options for definitive therapy of localised PCa.

RADICAL PROSTATECTOMY FOR LOCALISED PROSTATE CANCER

Theodor Billroth performed the first RP for PCa as perineal approach in 1869 [12]. The modern retropubic approach was proposed by Terrence Millin who reported a series of 20 cases who underwent RP due to urinary obstruction [13]. It soon became evident that postoperative 30-day mortality was relatively high and risk of incontinence and impotency was significant [14]. Through meticulous anatomical studies of the prostate and the nerves surrounding the gland, Patrick Walsh and Herbert Lepor refined the RP to include a nerve-sparing technique, which increased the change of regaining erectile function [15, 16]. These studies fuelled a rapid improvement in early and late complications rates following RP. Zincke and colleagues were one of the first groups to report these improvements in a large cohort of 3170 men who underwent RP during the 80'ies with a follow-up of up to 15 years [17]. With technological advances in surgical urology, first laparoscopic RP and later robot-assisted laparoscopic RP were introduced as feasible surgical techniques. Also, advances in anaesthesiology and postoperative care have positively affected the morbidity after RP. Today, RPs can be performed with a one-day hospitals admittance and minimal risk of postoperative mortality and early complications [18].

The indication for nerve-sparing RP remains unclear. The original anatomical studies demonstrated that nerve-sparing RP did not compromise cancer control, when performed in organ confined (pT2) tumours. The ability to predict specimen organ confined disease from preoperative evaluation has since been investigated in several studies and today a number of nomograms are available that can aid decision making about nerve-sparing surgery prior to RP [19]. Most studies report a careful selection of patients for nerve-sparing RP with primarily low- and intermediate risk patients elected for the procedure [20-22]. Only a few studies have evaluated whether nerve-sparing RP in itself increases the risk of PSM. The maintenance of erectile function is not only a matter of nerve-sparing surgery. Preoperative erectile function, age, and co-morbidity are some of the strongest prognostic factors for postoperative recovery of erectile function [23, 24]. Further, recent studies suggest that nerve-sparing surgery might increase the chance of quick recovery from postoperative incontinence, which ultimately could widen the indication for nerve-sparing RP [25]. Also, new anatomical studies have challenged the original understanding of the penile neural innervation and proposed new techniques of nerve-sparing RP which further questions the optimal selection of candidates for nerve-sparing RP [26].

A vast number of articles have described outcome after RP. However, no clear consensus exists when reporting surgical and oncological outcome after RP. Surgical outcome include short- and long term complications and histopathological features such as positive surgical margin rates (PSM). The ISUP advocates, and have published guidelines for, uniform interpretation of prostate pathology. However, there are still no standardised pathology protocols for handling of RP specimens [27].

The most frequently reported oncological outcome after RP is PSA recurrence, whereas studies reporting time to progression, metastasis, and death are fewer [28]. According to the PCa guidelines of the American Association of Urology at least 166 definitions of biochemical recurrence after definitive therapy exist in the literature whereof 56 definitions have been proposed for PSA recurrence after RP [29]. Therefore, comparison of RP series is complicated by definition of endpoints, pathological assessment and selection of patients.

The question whether RP is an effective treatment, i.e. reduce the risk of PCa death, has been investigated in three randomised trials. The Veterans Administration Cooperative Urological Group randomised 142 patients to RP versus expectant management [30]. Although survival favoured RP patients, the study did not have the statistical power to draw valid conclusions. The Scandinavian Prostate Cancer Group (SPCG) randomised patients with age≤75 years and clinically localized non-metastatic PCa (≤T2, N0, M0) and a life expectancy of >10 years to either RP or watchful waiting. Initiated in 1989, the SPCG-4 study randomised 693 patients from 14 centres during a 10-year period. After a median follow-up of 11 years the average absolute reduction in risk of PCa mortality was 6.1% for RP patients compared to watchful waiting [31]. The Prostate Cancer Intervention versus Observation Trial (PIVOT) was initiated in 1994 and randomised similarly to the SPCG-4 study, although with the significant notion that patients accrued for the PIVOT study had been diagnosed through PSA screening. The PIVOT study failed to show superiority of RP compared observation [32].

PROSTATE CANCER EPIDEMIOLOGY: A DANISH PERSPECTIVE.

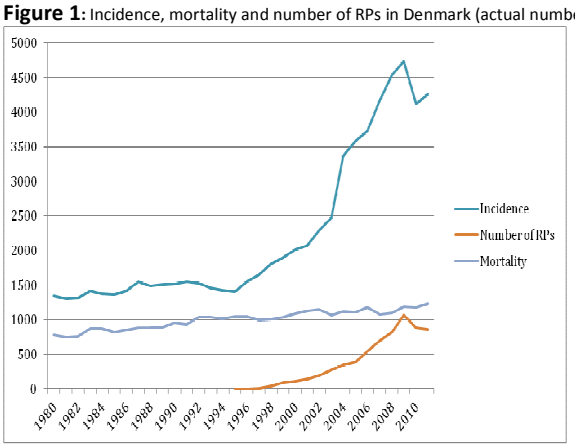
During the last three decades the worldwide epidemiology of PCa has changed dramatically. Excluding skin cancer, PCa is now the most common male cancer-diagnosis in the World [33]. An average annual increase in incidence of 4-8% in high resource countries has been observed. This increment has been attributed to the widespread use of PSA testing and increased awareness of PCa. Within the last 10 years PCa mortality rates have been slowly declining in some countries although it is still unclear to what extent this is explained by the early detection and treatment of PCa as a result of PSA testing [34].

The introduction of RP in Denmark in 1995 marked a milestone in Danish PCa epidemiology. For more than 25 years, the age standardised incidence rate of PCa had remained stable at approximately 70 per 100.000 men. From 1995 and onwards the incidence rate rose approximately 7.2% per year and in 2010 the age-standardised incidence rate had reached 163 cases per 100.000 men. Already in 2000, PCa became the most commonly diagnosed cancer-disease (ex. skin cancer) in Danish men and in 2010 PCa comprised approximately 25% of all cancer diagnosed in men. Although the Danish Urological Society has recommended a conservative approach to the use of PSA as early marker of PCa, the use of PSA testing at general practitioners in Denmark have increased dramatically and undoubtedly the increment in incidence can primarily be attributed to opportunistic PSA testing [35]. Interestingly, the Danish mortality rate of PCa has not changed for 35 years. The age standardised mortality rate remains at approximately 48 men per 100.000 men despite the marked changes in diagnosis and treatment. The rate corresponds to approximately 1100 men dying of PCa every year. The stable mortality rate has evoked debate since Denmark is the only Nordic country that has not experienced a decrease in PCa mortality [36].

TREATMENT OF LOCALIZED PROSTATE CANCER IN DENMARK.

The Danish health care system offers free and equal access to all health care services, including cancer treatment. The changes in Danish PCa epidemiology have had a huge impact on the health care system. Between 2000 and 2007, the annual number of outpatient visits for men with a PCa diagnosis increased from 16.898 to 62.992, and the number of men receiving any kind of treatment for PCa increased from 5917 to 13.399. As expected, the sharp rise in incidence has increased the number of men with localized PCa, and as a consequence, the treatment landscape of PCa in Denmark has changed accordingly. The number of men undergoing RP in Denmark has followed the increment in incidence closely:

Figure 1: Incidence, mortality and number of RPs in Denmark (actual numbers)



According to national registries, the number of men receiving any kind of radiation therapy for PCa, i.e. including palliative radiation, increased from 805 to 1547 between 2003 and 2010. A local registry at Rigshospitalet has documented that the number of men undergoing definitive external beam radiation therapy for localised and/or locally advanced non-metastatic PCa increased from 13 per year in 2000 to 113 per year in 2010 [37]. A similar trend in the rest of the country may be assumed.

The surgical and oncological outcome following RP in Denmark has only been addressed in oral presentations and publications based on small populations [38-41]. We initiated a PhD-study to investigate outcomes following RP from a large Danish cohort of patients who have been consecutively operated and prospectively followed in the Department of Urology, Rigshospitalet.

HYPOTHESES

Study I

Biochemical outcome after RP at Rigshospitalet according to the D'Amico risk classification model is similar to international reported results.

Study II

RP for D'Amico high-risk PCa at Rigshospitalet is safe and can cure a significant amount of patients without need for adjuvant therapy.

Study III
The location and number of PSMs are associated with risk of biochemical recurrence after RP.

Study IV
Apical PSM in pT2-tumours are not associated with an increased risk of biochemical recurrence after RP.

Study V
Nerve-sparing surgery is associated with an increased risk of PSM at Rigshospitalet

Study VI
PSMs in pT2-tumours are associated with a significant risk of biochemical recurrence and nerve-sparing surgery in pT2 tumours increase the risk of PSM.

MATERIAL AND METHODS

THE DATABASE

The first RP was performed at Rigshospitalet the 11th of August 1995. A simple database in paper format prospectively registered information on all relevant pre- and postoperative data in the early years. In 2005 the registry was changed to a modern web-based html-style database. In mid-2006 this database was functional and has been used ever since.

THE LOCAL RP PROTOCOL

To a large extent, RP has been centralized to few hospitals in Denmark. Rigshospitalet is a large university referral hospital in Denmark with the eastern part of the country, i.e. Zealand, Bornholm but also The Faroe Islands and Greenland as uptake areas.

Diagnostic work-up in men suspected for PCa has followed national guidelines from the Danish Urological Society/Danish Prostate Cancer Group. Trans-rectal ultrasound with guided biopsies of the prostate has been recommended for men with a PSA > 4 ng/ml and/or clinically suspected PCa based on digital rectal examination. Biopsies have been performed with 18 gauge needles. Until 2004, guidelines recommended that a standard biopsy set consisted of 6 cores: 2 apical, 2 mid-prostatic, and 2 from the base of the prostate. In 2005, the revised guidelines recommended a minimum of 10 biopsies: 2 apical, 4 mid-prostatic, and 4 from the base of the prostate.

Since 1995, a local guideline for preoperative work-up and treatment of localised PCa has been available at Rigshospitalet. According to these guidelines patients with clinically localized PCa and a life expectancy of more than 10 years are eligible for RP. Life expectancy is evaluated based on co-morbidity and assessed by each individual surgeon. All patients with PSA >10 ng/ml and/or biopsy GS ≥7 undergo a bone scan prior to RP to rule out metastatic (M1) disease. Staging evaluation includes bone scan, abdominal computed tomography and for selected patients, magnetic resonance imaging. Patients were staged according to UICC's TNM classification 2002 (6th edition) [42]. Patients undergoing RP prior to 2002 were re-classified according to the description of the digital rectal examination in the patient file. Open retropubic RP are performed according to the method described by Walsh (currently performed by 6 surgeons). Robotic assisted laparoscopic surgery (RALP) (DaVinci®) was introduced in 2009 and is performed by 2 of the 6 surgeons. Lymphadenectomy is performed in patients with biopsy GS≥7 and PSA≥10 ng/ml or with macroscopically suspicious lymph nodes. A standard lymphadenectomy is performed in the triangular space between the pubic bone, the external iliac vein and obturator nerve. Nerve-sparing surgery is offered to selected patients with preoperative self-evaluated erectile function sufficient for coitus. Unilateral nerve-sparing are performed in patients with cT1-cT2a/b, no tumour in apical biopsies on nerve-sparing side, PSA<10 mg/ml and biopsy GS 3+4 with a maximum of three positive biopsies on the nerve-sparing side. Bilateral nerve-sparing was performed only in patients with non-palpable disease.

Three months neoadjuvant endocrine therapy with one depot of gonadotropin-realeasing hormone agonist was used routinely in the first years after introduction of RP. Early reports indicated that neoadjuvant hormonal therapy reduced blood loss, BR-, and PSM-rates after surgery [43]. As a later randomized trial could not demonstrate any difference on BR rates, neoadjuvant hormonal therapy was abandoned in our institution [44]. Neoadjuvant hormonal therapy influences the assessment of final histopathology [45]. Therefore, specimen histopathology (pT, pN categories and PSM) is not reported for those patients.

Postoperatively, patients have been followed with PSA measurements every 3 months for the first year, thereafter twice a year for two years and then once annually. Biochemical recurrence (BR) is defined as the first PSA ≥ 0.2 ng/ml. No standardized PSA cut-off for performing bone scans at rising postoperative PSAs has been employed. Evaluation of suspected metastatic disease is performed according to the treating physician's discretion. Cause of death is determined from patient files and/or autopsy reports.

Patients with node-positive disease (N1) after RP receive immediate castration therapy according to our guidelines. No other patients receive hormonal or radiation therapy before BR or clinical progression is confirmed. Based on specimen histopathology and post RP PSA dynamics, we attempt to classify patients with BR as having either distant failure or local recurrence. Patients with local recurrence are treated with salvage RT and short-term (3 months) endocrine treatment. Patients with distant failure are offered endocrine therapy if PSA exceeds 20 ng/ml or the PSA doubling time is less than 1 year.

HISTOPATHOLOGICAL ASSESSMENT.

After fixation in 4% buffered formalin, RP specimens are inked for optimal orientation during microscopic examination (anterior surface in blue, posterior surface in black). Slicing is performed to ensure laterality, orientation in apical-basal direction and representation of the resection margin of the total prostate gland. The tissue is paraffin embedded and cut in 3-4 µm tissue sections, and the slides are stained with haematoxylin and eosin. When necessary, immunohistochemical staining for p63, high molecular weight cytokeratin (CK34βE12) and racemase P504S (after 2008) is performed. The surgical margin is considered positive if invasive prostatic glands are located at the inked margin. The location of PSM is reported as accurate as possible. The location of PSM is defined as apical when including the most apical, sagitally sectioned slide and the first horizontal section and non-apical when including the most basal, sagitally sectioned slide. Systematic reporting of margin location was implemented during 2006. Specimen handling has changed over time. At the beginning whole mount sections was used for microscopically evaluation.

During 2001-2002 this process was changed to partial embedding to facilitate a faster evaluation of the specimens[46].

PATIENT POPULATIONS, STUDY DESIGNS AND STATISTICAL ANALYSIS.

Detailed descriptions are available in the accompanying manuscripts. In the following section, a short summary of objectives, study design and analysis is presented.

Table 1: Overview of patient populations

Study	N	Period	Median F/U	Inclusion
I	1200	95-2010	4 years	All
II	231	95-2010	4.4 years	High-risk
III	605	07-2009	2.7 years	All
IV	1133	95-2011	3.6 years	pT2 tumours
V	1148	06-2011	N/A	All
VI	1133	95-2011	3.6	pT2 tumours

Study I

The objective of this study was to describe biochemical outcome following RP at Rigshospitalet. An analysis of BR rates in the consecutive cohort of the first 1200 patients who underwent RP between 1995 to the beginning of 2010 was conducted. BR was defined as the first PSA≥0.2 ng/ml (unfortunately incorrectly given as >0.2 ng/ml in manuscript). In order to more accurately compare our results to other institutional series, patients were stratified according to the D'Amico classification:

Table 2: D'Amico risk classification

Low-risk
PSA <10 ng/ml **and** biopsy Gleason score ≤6 **and** cT1 or cT2a

Intermediate-risk
PSA ≥10 **or** < 20 ng/ml **or** biopsy Gleason score 7 **or** cT2b

High-risk
PSA ≥20 ng/ml **or** biopsy Gleason score ≥ 8 **or** cT2c

In analysis of BR, a total of 22 patients were excluded; 18 with N+ disease, three with pT4 M+ disease and one with T0 disease. Time to BR was analysed in Kaplan-Meier estimation and Cox proportional hazard model and calculated from the date of surgery. Further, the risk of BR was analyzed in multivariate modelling including age, cT-category, open vs. Robotic technique, biopsy GS and PSA. PSA was treated as a categorical variable in 4 intervals (<=4, 4.1-10, 10.1-20, >20) in this analysis.

Study II

The objective of study II was to perform an in-depth analysis of outcome following RP for D'Amico high-risk disease. Patients operated between 1995 and end of 2010 were included. The endpoints of interest were biochemical- and metastasis-free survival, cancer-specific and overall survival. A total of 231 patients were included in the analysis. Time to BR was calculated from the date of surgery to a postoperative PSA ≥0.2 ng/ml. Patients with N+ disease (10 men) at RP was excluded from analysis of BR as these patients received immediate androgen deprivation. Time to metastatic disease was calculated from the date of surgery to the first bone scan with pathological uptake to the bone interpreted as metastatic disease. Survival analysis was done using Kaplan-Meier estimation and Cox regression modelling. Due to limited number of events, multivariate modelling was limited to risk of BR. Two separate multivariate models for pre- and postoperative parameters were done. In this analysis, PSA was analysed as a continuous variable and entered on a logarithmic base 2 scale. Therefore, hazard ratios (HR) for PSA represent a two-fold difference in PSA.

Study III

The objective of this study was to investigate if the location and number of PSM influence the risk of BR after RP. The hypothesis was that patients with apical PSM have a low risk of BR as the PSM is left as devitalised tissue due to ligation of the DVC and electro-coagulation in the plane between the DVC and prostate during RP. Further, we hypothesized that two or more PSM significantly increase the risk of BR. The number of PSMs was categorized into two groups: 1 vs. ≥2. The location of PSM was classified as apical or non-apical. The apical group included PSM found exclusively at the apex. To avoid pathological issues regarding the revised version of the ISUP 2005 guideline we only included RPs from 2007 through 2009 – a total of 605 patients. The primary endpoint was BR defined as the first PSA ≥0.2 ng/ml (incorrectly given as >0.2 ng/ml in the manuscript). N+ patients (N=6) were excluded from the analysis of BR. Biochemical recurrence-free survival was calculated using Kaplan-Meier analysis with stratification for both location and number of PSM. Survival curves were compared using log-rank statistics. In multivariate modelling the risk of BR was analysed using PSA (log base 2), pT-category, specimen GS, PSM status, PSM location and PSM number.

Study IV

Based on an exploratory analysis of pT2 tumours from study III it was decided to investigate PSM location and its impact on BR in all pT2 tumours in patients operated from 1995-2011. To adhere to the revised ISUP 2005 guidelines all pT2 specimens where PSM location had not been reported were re-reviewed. In total, 1133 patients with pT2 tumours were included in this analysis. PSM location was stratified into apical and non-apical PSM accordingly. The primary objective of this study was to analyse risk of BR in patients with pT2 PSMs, and secondly to analyse whether the location of PSM influenced the risk of BR. BR was defined as the first PSA≥0.2 ng/ml after RP. No patients received adjuvant therapy until BR was confirmed. Time to BR was calculated from the date of surgery. Kaplan-Meier estimation was used for univariate analysis of biochemical recurrence-free survival. Multivariate analysis was done using Cox proportional hazard model, including cT-category, biopsy and specimen Gleason score (GS), PSA (log base 2), percent biopsies with cancer (percent positive biopsies, PPB), surgeon, nerve-sparing technique and type of surgery (robotic vs. open). PPB was calculated from the number of positive cores divided by the total number of cores, and HR represents a 10% difference in PPB. HR for age represents an increase per 10 years. For categorical covariates, cT1, biopsy GS≤6, surgeon A (most procedures), open surgery, non nerve-sparing surgery, and negative margin status was entered as references.

Study V

Based on empirical data it was speculated that nerve-sparing surgery increases the risk of PSM at our institution. Therefore, an analysis of the consecutive group of patients who underwent RP between 2006 and 2011 were performed. This period was chosen

to avoid issues with the ISUP 2005 revised guidelines. A total of 1148 patients were included in the analysis, whereof 332 (28.9%) patients underwent nerve-sparing surgery. The primary objective of this study was to assess risk factors associated with PSM. Secondary objective was to describe the location of PSM (left, right, bilateral) and it's relation to nerve-sparing technique. Multivariate logistic regression analysis for risk of PSM was performed, including cT-category, biopsy GS, PSA (log base 2), percent positive (for cancer) biopsies (PPB), surgeon, nerve-sparing technique and type of surgery (robotic vs. open). Results are presented by the OR with 95% CIs. For categorical covariates, cT1, biopsy GS≤6, surgeon A (most procedures), open surgery and wide resection was entered as references.

Study VI

Based on data from study V an analysis of risk factors associated with PSM in pT2 tumours was planned. The objective was to examine if nerve-sparing surgery in organ-confined tumours increase the risk of PSM and BR. Secondly, it was sought to identify the optimal candidates for nerve-sparing surgery. In this study, all pT2 tumours that were not described according to the ISUP 2005 guideline were re-reviewed. This analysis included 1133 patients. Multivariate logistic regression analysis for risk of PSM was performed, including cT-category, biopsy GS, PSA (log base 2), percent positive (for cancer) biopsies (PPB), surgeon, nerve-sparing technique and type of surgery (robotic vs. open). To account for low surgical volume/learning curve, surgeons who had performed <10% of the RPs were excluded from multivariate analysis. To account for preoperative selection of patients for nerve-sparing surgery, patients with cT3 and biopsy GS>= 4+3 were excluded. To identify optimal candidates for nerve-sparing surgery, in whom the technique will not increase the risk of PSM, we performed a series of multivariate logistic regression analyses, trying to adjust for the preoperative skewed distribution of tumour characteristics between patients undergoing nerve-sparing and non nerve-sparing surgery. The objective was to identify a group of patients by preoperative parameters where the OR was close to one, indicating that nerve-sparing surgery per se after multivariate adjustment did not increase the risk of PSM compared to non-nerve-sparing surgery.

BR was defined as the first PSA≥0.2 ng/ml after RP. Risk of BR was analysed in univariate analysis using Kaplan-Meier estimation. Multivariate analysis was done using Cox proportional hazard model.

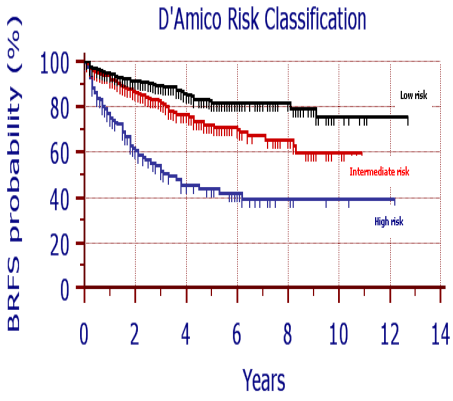
RESULTS

Descriptions of patient characteristics and pathology data are detailed in the accompanying manuscripts. In the following section, the core results according to the hypothesis and objectives are presented.

Study I

During a median follow-up of 4 years a total of 214 (18%) out of 1200 patients experienced BR. This corresponded to an estimated 5- and 10-year overall biochemical recurrence-free survival (BRFS) of 71.7% (95CI:67.9-75.4) and 63.2% (95CI%:56.9-69.5), respectively. BRFS according to the D'Amico risk classification is depicted here:

Figure 2: Biochemical recurrence-free survival, study I.



	N	Estimates of BRFS following RP 5 years % (95%CI)	10 years
Low-risk:	166	81.6 (76.4-86.7)	75.3 (65.3-85.2)
Int-risk:	573	71.9 (65.9-77.8)	59.7 (49.3-70.1)
High-risk	414	43.9 (34.1-53.7)	39.3 (28.6-50.0)

In multivariate analysis we demonstrated that risk of BR is associated with PSA>10, biopsy GS≥8, and ≥ cT2 tumour at diagnosis:

Table 3: Multivariate analysis for risk of biochemical recurrence (reduced model)

	HR	95%CI	P-value
PSA 10-20	1.7	1.2-2.3	<0.001
PSA ≥20	2.8	1.9-4.2	<0.001
Biopsy GS 8-10	3.7	2.4-5.6	<0.001
cT2	1.6	1.2-2.2	<0.001
cT3	2.6	1.5-5.6	0.0034

Reference: PSA 4-10, biopsy GS≤5, T1c
Model included: age, PSA, biopsy GS, robot vs. open surgery, cT-category

Study II

During a median follow-up of 4.4 years BR occurred in 95 of the 231 high-risk patients included. Of these 95 patients, BR was interpreted as distant failure in 51 patients and as local recurrence in 32 patients. Twelve patients were not classified at the time of follow-up. Metastatic disease occurred for 18 patients and 17 patients died; nine of them due to PCa. The estimated 10-year survival probabilities for endpoints of study II was:

Table 4: 10-year survival probabilities

	Estimate	**95%CI**
BRFS	49%	40-57%
Metastasis-free survival	85%	76-92%
Overall survival	84%	73-91%
Cancer-specific survival	90%	79-95%

In multivariate analysis with preoperative parameters, biopsy GS ≥8 were significantly associated with increased risk of BR compared to biopsy GS 6, HR=4.1 (95%CI: 2.2-7.4, p<0.001). When modelling postoperative parameters, pT- category was the

strongest predictor of BR. As an example, pT3b tumours significantly increased the risk of BR compared to pT2 tumours, HR=5.3 (95%CI:2.9-9.6, p<0.001).

Study III

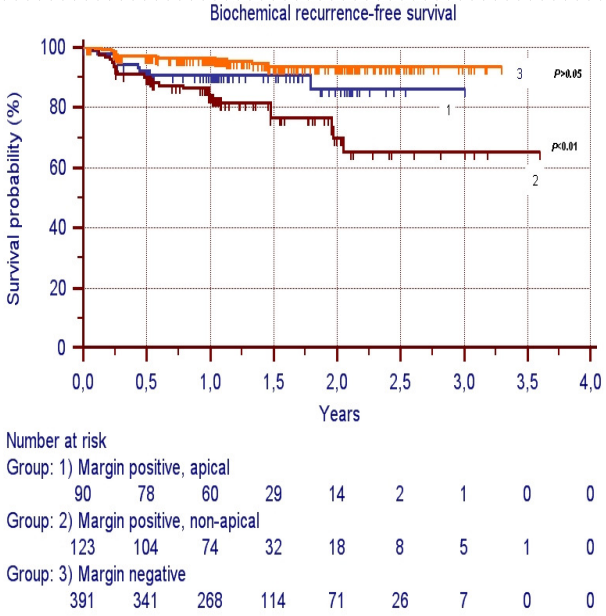
The median follow-up was 2.7 years in study III. The key histopathological findings were:

Table 5: Pathological findings in 605 patients

	N	%
Overall PSM	214	35.4
pT2 PSM rate	128	28.1
Apical PSM	91	53.9
Non-apical PSM	123	57.5
One PSM	177	82.7
≥2 PSM	37	17.3

A total of 81 patients experienced BR during follow-up. The estimated 3-year BRFS was 97.7%, 85.4% and 66.9% for margin negative patients, apical PSM, and non-apical PSM patients; respectively.

Figure 3: BRFS stratified on margin location, study III



Stratified on the number of PSMs, there was a significant difference in 3-year BRFS for 1 vs ≥2 PSMs (77.2% vs. 57.4%, log-rank p-value=<0.001). In multivariate analysis, including age, PSA, specimen GS, and pT-category, the difference in risk of BR according to location and number of PSM disappeared. In multivariate analysis of all patients, the risk of BR in apical and non-apical PSM was identical. However, in an exploratory multivariate analysis only including pT2 tumours the location of PSM had a significant impact on risk of BR. Compared to margin negative patients; non-apical pT2 PSM patients had 3.4-fold significant increased risk of BR, whereas apical pT2 patients had a 2.1-fold insignificant risk BR.

Study IV

Median follow-up in study IV was 3.6 years. The overall rate of PSM in pT2 was 26.3%. The distribution of PSM location was 49.7% apical and 50.3% non-apical. There were no differences in preoperative characteristics between apical and non-apical PSMs. Overall, the 5- and 10-year BRFS survival was 88.6% (95%CI:86.2-91.0) and 76% (95%CI:69.7-82.2). In univariate Kaplan-Meier analysis there was a trend (log-rank p-value = 0.09) for difference in BRFS between apical and non-apical PSM patients. Multivariate analysis demonstrated a significant impact of location of PSM on risk of BR compared to margin negative patients:

Table 6: Cox proportional hazard model for risk of BR.

	HR	95%CI	*p*
Age, per increasing 10 years			
	0.9	0.6-1.2	0.4
PSA, for every doubling			
	1.5	1.2-1.9	<0.001
pT-category			
pT2a/b (ref)	1		
pT2c	1.3	1.0-1.7	0.02
Specimen Gleason score			
<=6 (reference)	1		
3+4	1.3	0.8-2.0	0.27
4+3	2.8	1.6-5.0	<0.001
>=8	5.6	2.7-12	<0.001
Margin location			
Margin neg (Ref)	1		
Apical	2.1	1.2-3.4	0.006
Non-apical	3.2	2.0-4.9	<0.001

However, there was only a trend for statistically significant difference in the adjusted risk of BR between apical and non-apical margins (p=0.08). The Cox-adjusted (model from table) cumulative hazard for BR is demonstrated here:

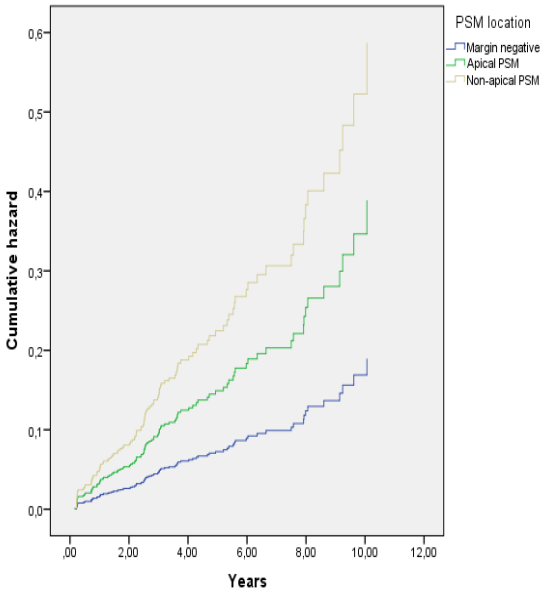


Figure 4: Cumulative hazard for BR, Cox-adjusted curves stratified on PSM location

Although not statistically significant different at 5% level, the adjusted cumulative hazard of BR for apical PSM patients was 12% compared to 22% for patients with non-apical PSMs.

Study V

Of the 1148 patients included in study V, a total of 332 patients underwent nerve-sparing surgery. The overall PSM-rate was 31.4%. As expected based on preoperative selection of patients for nerve-sparing RP, statistically significant differences in cT-category, PSA, percent positive biopsies and biopsy GS favouring patients undergoing nerve-sparing RP were found. Interestingly, the PSM-rate did not vary significantly between wide resection, unilateral- and bilateral nerve-sparing surgery but remained approximately 30% in the three groups. When sub-stratifying margin location on side of unilateral nerve-sparing procedure an identical rate of ipsi- and contralateral PSM was found:

Table 7: Nerve-sparing surgery and side of PSM, study V

Site: N (%)	Wide resection	Unilateral NS left	Unilateral NS right	Bilateral NS
Negative margin	551 (67.5)	78 (70.9)	75 (69.4)	83 (72.8)
Right-sided PSM	116 (14.2)	15 (13.6)	13 (12.0)	18 (15.8)
Left-sided PSM	79 (9.7)	11 (10.0)	17 (15.8)	8 (7.0)
Bilateral PSMs	70 (8.6)	6 (5.5)	3 (2.8)	5 (4.4)

Abbreviation: NS= nerve-sparing

When analyzing risk factors associated with a PSM in a multivariate logistic regression analysis, PSA, percent positive biopsies, nerve-sparing surgery and surgeon was demonstrated as primary factors associated with risk of PSM. The key findings from the model were:

Table 8: Risk for positive surgical margin, key findings from logistic regression.

Variable	OR	95% CI	*p*
Biopsy Gleason score			
≤6 (ref)	1		
3+4	1.42	1.0-1.9	0.03
4+3	1.44	0.9-2.4	0.15
≥8	1.64	0.9-3.0	0.12
N/A*	1.39	0.5-3.8	0.50
PPB	1.11	1.0-1.2	0.002
PSA, ng/ml	1.56	1.3-1.9	<0.0001
Surgical technique			
Non nerve-sparing (ref)		1	
Nerve-sparing	1.50	1.0-2.1	0.03
Surgeon			
A (ref)	1		
B	1.65	0.98-2.8	0.06
C	0.71	0.5-1.0	0.07
D	0.50	0.3-0.8	0.01
E	0.54	0.3-0.8	0.01
F	1.54	0.7-3.0	0.27

Study VI

The cohort is identical to the cohort in study IV, including all pT2 patients who underwent RP between 1995 and 2011. Median follow-up was 3.6 years. A total of 375 (33.1%) underwent nerve-sparing RP. Again, as a result of the selection criteria, a significant differences in distribution of preoperative characteristics in favour of patients undergoing nerve-sparing RP was found. In multivariate logistic regression modelling, nerve-sparing surgery was associated with a 68% increased risk of PSM.

Table 9: Risk of positive surgical margin in pT2 tumours (reduced model).

	OR	95%CI	P-value
PSA, for every doubling			
	1.8	1.3-2.0	<0.001
Surgeon			
A (reference)	1		
B	0.97	0.6-1.5	0.9
C	0.81	0.5-1.4	0.4
Procedure			
Non nerve-sparing(ref)	1		
Nerve-sparing	1.68	1.1-2.5	0.01

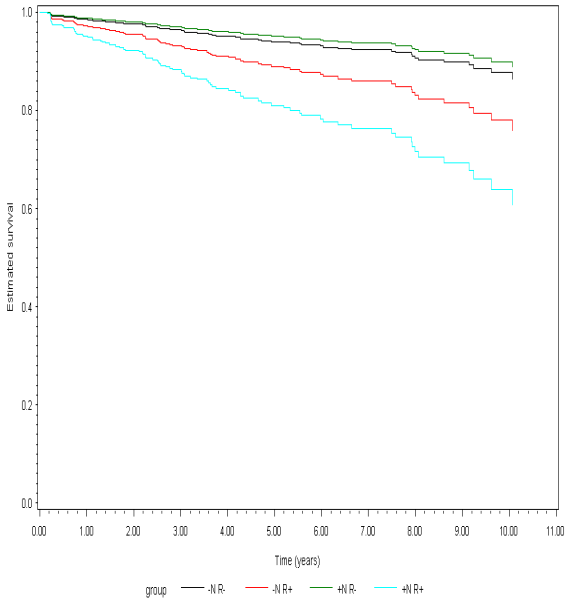
Excluded: 3 surgeons (D, E, F), biopsy GS=N/A, 4+3 and >=8, cT-category=T3 to adjust for skewed distribution of preoperative parameters.

In a series of logistic regression analyses, we identified patients with T1, biopsy GS≤6 and PSA≤10 ng/ml to have identical risk of PSM compared to patients undergoing non nerve-sparing RP.

Overall, the estimated 5- and 10-year biochemical recurrence-free survival (BRFS) of 88.6% (95%CI:86.2-91%) and 76% (95%CI:69.7-82.2%), In multivariate analysis, a pT2 PSM was associated with a 2.4-fold increased risk of BR (HR=2.4, 95%CI:1.6-3.6; p<0.001) compared to margin negative pT2 tumours. Nerve-

sparing surgery was not independently associated with an increased risk of BR. However, a borderline statistically significant interaction between NS surgery and pT2 PSM was found (p=0.08). When adjusting for PSA, biopsy GS and cT-category this interaction had an impact on the risk of BR. Patients that underwent non nerve-sparing surgery with pT2 PSM had an 1.9-fold increased risk of BR compared to margin negative patients (HR=1.9, 95%CI: 1.1-3.2). Patients who underwent nerve-sparing surgery and was found to have pT2 PSM had an 4.2-fold increased risk of BR compared to nerve-sparing patients with negative margin status (HR=4.2, 95%CI:1.9-9.1). This interaction is shown in a Cox-adjusted Kaplan-Meier analysis of BRFS. A typical nerve-spared patient (cT1, biopsy GS=6 and PSA=7) with a PSM have a worse BRFS compared to a non nerve-spared patient with PSM.

Figure 5: Cox-modeled BRFS for the interaction nerve-sparing surgery and PSM



Abbreviations: -/+N= non- and nerve-sparing surgery. -/+R= negative and positive margin

DISCUSSION

The focus of this thesis was to report a compilation of surgical and oncological results following RP for localized PCa at our institution. The predetermined objective was to report risk factors associated with biochemical outcome, and to analyse the relationship between nerve-sparing RP and risk of PSM.

In 2009, we reported the first results from our database, focusing on 30-day morbidity and mortality [47]. In the first consecutive 719 patients (1995-2007) results demonstrated that 164 (22.8%) experienced one or more complication following RP with only severe complications in 1.1% of the patients. One patient died within 30 days of surgery. Moreover, statistically significant reductions in median operating time, number of blood transfusions required, and length of hospitalization had occurred over time, reflecting increasing surgical experience and optimization of the postoperative course. These results seem comparable to other single-institutional series from other academic centres; although the comparison should be interpreted with caution as there is a significant variation in reported frequencies of complications after RP in the literature [17, 48]. Interestingly, the paper also demonstrated that during the first 12 years, the median preoperative PSA decreased (12.8 to 9.5 ng/ml), the median preoperative age increased (61 to 64 years), and the proportion of patients with cT1 tumours increased to encompass approximately 55% of all patients undergoing RP.

Recently, we reported survival among the first 1350 consecutive patients. During a median follow-up of 3.4 years, 59 patients died, 17 of these from PCa. This results in an estimated ten-year overall survival of 89.3% (95%CI:95.8-92.8%), and ten-year cancer-specific survival was 96.6% (95%CI:94.7-98.5). Acknowledging the short median follow-up, these results seem comparable to other series [28].

General considerations

Direct comparison of results across surgical series is complex. A prominent cause for misinterpretation is selection bias, including sampling bias and indication bias.

The diagnostic strategy for PCa in the general population will influence tumour characteristics in a cohort undergoing RP. As demonstrated in European Randomised Study of Screening for Prostate Cancer (ERSPC), PSA screening induce a lead-time in PCa diagnosis of a mean of 6 years as reported in the [49]. As a result of this, PSA screening induce a significant stage migration in patients eligible for RP [50]. The decline in median preoperative PSA at our institution, the increased incidence of PCa in Denmark, and increase in number of PSA tests performed by general practitioners, indicate that opportunistic PSA testing to some extent is practiced also in Denmark [35]. Still, PSA based screening is not recommended officially in Denmark, and therefore we have argued that our results should primarily be compared to surgical series from the early PSA era.

Tumour characteristics in patients undergoing RP are also affected by biopsy strategy. According to national Danish PCa guidelines the indication for biopsy of the prostate has remained unchanged since 1995 with a PSA biopsy threshold of 4.0 ng/ml and/or a suspicious digital rectal examination. Until 2001, a standard set of biopsies included six cores. Since then, biopsy practices have gradually changed and today it is recommended to perform at least 10 cores. Increasing the number of cores increase the likelihood of finding cancer, that in turn is likely to induce stage migration in patients referred for RP [51].

The general indication for RP has not changed in our institution since 1995. Clinically localised PCa and a life expectancy of at least 10 years have been the key criteria when selecting patients for RP. However an increase in median age at surgery is indicative of some drift in indication. Interestingly, opportunistic PSA testing in the background population would have been expected to decrease age at surgery due to lead-time. It is likely that the increasing age at surgery primarily is a reflection of the longer life expectancy that increased by approximately 3 years for

men age 45-75 from 1995 to 2010 [52]. The delay of RP from 1-4 years in the increasing number of patients initially managed by active surveillance may also play a role [53, 54].

OVERALL STRENGTHS AND LIMITATIONS

Relatively short follow-up (range 2.7-4.4 years) and lack of maturity limit the strength of our statistical analyses despite the large sample sizes. All data analysis has been performed, or supervised, by a statistician to ensure internal validation of the models used. However, external validation of statistical models, especially investigating the association of nerve-sparing surgery with PSM, seems warranted.

This patient cohort is heterogeneous, which is both a strength and a limitation. The cohort is strictly consecutive but the patients have been enrolled at a time where the Danish PCa epidemiology has changed. Changes in preoperative characteristics over time have not been adjusted for. To some extent we have adjusted for this by performing time-dependent sensitivity analysis in the Cox-regression models as internal validation. None of these tests have so far indicated a potential time-dependent interaction. Further, although one surgeon performed approximately 50% of the RPs, the total number of surgeons (6) is high compared to many published reports and also reflects training of new surgeons. Acknowledging the importance of surgical routine and the existence of learning curves, this may have affected outcome, especially in terms of PSM rates [55]. We tried to balance for this by excluding low-volume surgeons in some of our analyses. Also, the majority of patients underwent open retropubic RP but also up to 150 robot-assisted laparoscopic procedures were included in the individual study cohorts. We decided to include robotic procedures to adhere to the consecutive nature of our analyses. However, the few robotic procedures that were included also represent a learning period and this could affect our results, especially in terms of PSM rates. We recently performed an in-depth analysis of our robotic procedures and demonstrated that PSM-rates have remained identical to open RPs when excluding the first quartile (58 procedures) as representing the learning curve [56]. Also, no comparative studies have so far demonstrated that robotic surgery is superior to open surgery in terms of oncological outcomes [57].

Another overall shortcoming is histopathology assessment. There has been a change in practice both in terms of specimen handling but also in terms of interpretation over time. The impact of total versus partial embedding on risk of missing PSM has been debated intensely in the past years [27, 58]. The change from whole mount sections to partial embedding at our institution could have affected histopathological reporting. It was not possible to compensate for this in our studies. Further, the introduction of ISUP 2005 guidelines undoubtedly changed the interpretation of Gleason score, also in our institution. As described we did not re-review all patients throughout the studies. However, our data is strengthened by the fact that a limited number of pathologists (5) have been involved in histopathological assessment over the studied period.

Compared to contemporary reports, in which PSM rates are as low as 10%, our PSM rate (approximately 30%) seems high [59]. Currently, there are no reports from other Danish centres for comparison. Compared to PSM rates in the PIVOT (22.8%), SPCG-4 study (35.3%) and early PSA era American series (e.g. Mayo Clinic: 33%) our PSM rates seem comparable [31, 32, 60].

A particular strength of our studies is the calculation of time to BR, which is not influenced by adjuvant treatment since none of our patients received any adjuvant treatment before BR was confirmed.

STUDY I-VI

In the following section the studies are discussed individually:

STUDY I

The D'Amico risk classification was proposed in a landmark paper in JAMA in 1998 [61]. The model categorizes patients in three groups according to their risk of BR after either RP, internal or external beam radiation therapy. Since then, the model has been externally validated in several RP papers and is now considered as part of everyday PCa terminology. Although each risk group has a certain degree of heterogeneity the model facilitates a reasonably balanced method of comparing results across surgical series. In study I we compared our BRFS to a selected group of high-volume papers:

Table 10: Comparing BRFS in Study I to other reports

Risk groups	Low	Intermediate	High
BRFS at		5/10 years	
D'Amico et al [61, 62]	85/83%	60/46%	30/29%
Boorjian et al [60]	90/82%	78/65%	68/55%
Hernandez et al [59]	95/90%	77/65%	55/45%
Røder et al. (Study I)	82/75%	72/60%	44/39%

Overall, our results seem comparable to the papers mentioned above. There are a number of plausible explanations for differences within each risk group. First, none of the papers used the same definition of BR. Secondly; especially the high-risk group is susceptible to selection and indication bias due to the individual institutional treatment policy. As an example, many institutions offer high-risk patients with the worst prognostic factors radiation therapy instead of RP. Also, high-risk patients is a heterogeneous group, i.e. patients with only one high-risk feature is likely to endure a lower risk of BR compared to a patient with all three features of high-risk disease.

Limitations of study I:

An overall strength is that the cohort is strictly consecutive and only to a limited extent influenced by selection. Only 26 patients with low-risk PCa and 131 intermediate-risk patients underwent external beam radiation therapy

between 2000 and 2010 at Rigshospitalet. However, the high-risk group (166 patients) is more selected, including only 33 with T3-disease. Of the 503 patients that underwent external beam radiation therapy in 2000-2010, 70% had T3 disease. Another limitation is a short follow-up, although 250 patients were followed for more than 5 years. Also, a total of 113 patients received neoadjuvant treatment and therefore were excluded for analysis of specimen pathology. However, we chose to include these patients in calculation of BRFS as *Aus et al.* have shown that 3-months neoadjuvant endocrine therapy prior to RP do not decrease risk of BR compared to RP alone [44].

STUDY II

One of the current dilemmas in management of high-risk localized and locally advanced non-metastatic PCa is the choice of primary therapy. Randomized trials of external beam radiation therapy have demonstrated improved survival for the combination of radiotherapy and endocrine therapy compared to either therapeutic modality alone in high-risk localized and locally advanced PCa [62-64]. Recently, *Vickers et al.* demonstrated that high-risk patients undergoing RP have an estimated individual absolute risk reduction of PCa mortality of up to 25% compared to observation based on data from the SPCG-4 study [8]. Whether this benefit can be improved further by the addition of adjuvant radiation therapy to patients with postoperative high-risk features is still unanswered.

In the absence of randomized trials comparing primary therapeutic strategies in high-risk disease we conducted updated and in-depth analysis high-risk patients who underwent RP at our institution. The results demonstrated that nearly 50% of the patients remain disease-free up to 10 years later. Thus, a significant proportion of patients seem to be spared the morbidity associated with years of endocrine therapy if treated with radiation therapy combined with endocrine therapy. Our results seem to concur with other reports although postoperative management of these patients vary significantly between reported series [60, 65, 66].

Limitations of study II:

Short follow-up and low number of events compromised statistical analysis of outcomes such as risk for metastasis and PCa death. There are some discrepancies between the patient populations in study I and II. The higher number of patients in study II reflects that almost one additional inclusion year was added between the two studies. Further, our database underwent several updates in the years 2008-2010 when Denmark switched to national electronic patient files. New relevant data was updated through patient file viewing, which affected risk groups slightly. Also, we upgraded the total number of patients who received neoadjuvant treatment from 109 to 123. However, only 21 of the high-risk patients had received neoadjuvant treatment and for those patients the median follow-up was 10.9 years, which is why we chose to include them in the analysis of BR and survival.

STUDY III+IV

Currently, there is no clear consensus about the use of adjuvant therapy for post RP high-risk patients. Extrapolating data from other cancers, such as breast and colorectal cancers [67, 68], adjuvant therapy could reduce the risk of recurrence in these patients. Three randomized trials have showed that post-operative radiation therapy prolongs time to BR compared to a wait-and-see strategy, translating into a survival benefit in one trial [69-71]. The timing of post-RP radiation therapy, i.e. adjuvant vs. salvage, is currently investigated in the RADICALS trial for pT2 tumors with PSM and any pT3 tumors after RP [72]. Also, docetaxel as adjuvant therapy in post-RP high-risk patients is currently investigated in the randomized AdPro (SPCG-12) trial.

However, there is no clear consensus about the definition of post-RP high-risk disease. A number of observational studies have demonstrated that patients with PSM are at a higher risk of BR compared to margin negative patients [28]. A large study have shown that extensive and numerous PSM increase the risk of BR compared to small and unifocal PSM [73]. Further, the location of PSM has been demonstrated to impact on biochemical outcome [73-77].

In study III+IV we investigated the significance of PSMs. Overall, a PSM increased the risk of BR 2-3 fold in multivariate analysis which concur with other reports [78]. Study III resulted in two important findings. First, location of PSM in univariate analysis had an impact on BRFS, which disappeared in multivariate analysis. This was interpreted as an effect of the interaction of between pT3 tumours and PSM and their effect on risk of BR, where the presence of pT3 dilutes the effect of PSM per se [74, 75]. Secondly, exploratory analysis in study III indicated that location of PSM in pT2 tumours affected the risk of BR with non-apical having a higher risk than apical PSM.

Two studies support our results that non-apical PSM are associated with increased risk of BR compared to apical PSM. *Blute et al* found a significantly lower 5-year BRFS (56%) in patients with margin involvement at the prostate base compared with patients with positive margins at the apex, anterior prostate or multiple sites (78-82%), also when adjusting for preoperative PSA and tumour grade. *Eastham et al* found that a PSM at the posterolateral and the posterior part of the prostate was associated with an increased risk of BR (HR=2.8 and HR=1.9, respectively) compared to negative margin status in a study including 201 margin positive patients.

Study III led to study IV where we, in an attempt to exclude the impact of pT3, focused on the prognostic importance of PSM in pT2 tumours exclusively. Although study IV failed to demonstrate a statistical significant difference in risk of BR at the 5% level between non-apical and apical PSM (p=0.08), a strong trend for a clinical relevant differ-

ence was demonstrated. Thus, an absolute difference of 10% (12% vs. 22%) in hazard ratio for BR at 5 years between apical and non-apical PSM was found in a Cox-adjusted analysis. In conclusion, results from study III+IV support the finding that location of PSM has an impact on the risk of BR, especially in pT2 tumours. This may have an impact on the future management of post RP patients.

Limitations of study III+IV

The main limitation is the simplistic separation of margin location into two categories. This was done in order to enhance statistical modelling. Also, we did not adjust for length of each PSM or number of PSMs in each specimen. This would potentially have diluted the effect of location on BRFS. External validation of the models used in these studies is critical. As a result of short follow-up and few events, we did not investigate the impact of PSM on other clinical relevant endpoints such as metastasis-free survival and death.

STUDY V+VI:

RP performed with nerve-sparing technique is a surgical procedure that essentially necessitates blind resection and division of tissue very close to the tumour-bearing organ. Therefore, proper selection of candidates for nerve-sparing RP is crucial. Although several nomograms aim to predict pT-category from preoperative parameters, most lack independent validation. The nomograms are constructed based on studies showing low PSA, low GS, and low cT-category to be associated with the likelihood of organ confined disease[19]. Per se, the selection of patients with low-risk tumour characteristics for nerve-sparing surgery would be expected to result in favourable outcomes, both in terms of PSM rates, and in BRFS. Only a few studies have investigated the risk of PSM associated with nerve-sparing RP with multivariate modelling [20, 21, 76, 79].

Study V confirmed the findings from other large surgical series - a similar rate of PSMs between nerve-spared and non nerve-spared patients was found [20, 21, 80]. Further, study V demonstrated that ipsi- and contralateral PSM were equally frequent in unilateral nerve-sparing RP. In logistic regression analysis, nerve-sparing RP at our institution was demonstrated to be associated with a relative 56% increased risk compared to non nerve-sparing RP. This has not been found by others. Study V also demonstrated an impact of the individual surgeon on risk of PSM as it has been demonstrated by others [55].

One of the caveats of study V is the possibility that the increased risk of PSM reflect poor selection of patients for nerve-sparing RP with preoperative understaging driving a bias in multivariate analysis, i.e. high number of patients with pT3 tumours who underwent nerve-sparing RP due to preoperative misclassification. To adjust for this, study VI was performed to analyse whether nerve-sparing surgery in pT2 tumours only increased the risk of PSM. In study VI, the risk of PSM for patients undergoing nerve-sparing RP was 68% higher relative to patients who underwent non nerve-sparing RP. Study VI also investigated whether the increased risk of PSM translated into higher risk of BR. A trend for an interaction between nerve-sparing RP and PSM on risk of BR was found. When computing this interaction in an adjusted Cox model it was demonstrated that a typical nerve-spared patient, i.e. T1, PSA=7, biopsy GS=6, endured a higher risk of BR if PSM was present compared to a similar patient with PSM despite undergoing non nerve-sparing RP. A cautious explanation of this result could be that nerve-sparing RP increase the risk of multifocal, extensive, and non-apical PSM, whereas apical PSM are more frequent after non nerve-sparing RP. In fact, in a univariate analysis there was a trend for an association between non-apical location of PSM and nerve-sparing surgery as showed by others [79].

Limitations of study V+VI

The optimal statistical modelling of the surgeon factor is complicated. In study V+VI, surgeons were treated as categorical variables with reference to the surgeon who had performed the most procedures and therefore to some extent account for individual volume and but only to a limited extent account for inter-surgeon variability. Further in-depth analysis of this is ongoing.

We did not evaluate unilateral and bilateral nerve-sparing procedures separately. This was done in order to enhance statistical modelling. However, comparing two groups that have a skewed distribution of risk factors prior to analysis is not without problems. Secondly, the accuracy of the models varied between an AUC of 0.6 to 0.7. Although this result is typical for logistic regression models in PCa it seems warranted to validate our results in another dataset.

SUMMARY

BACKGROUND:

RP for localized PCa was introduced at Rigshospitalet in 1995. Since then, the incidence of PCa and number of RPs performed every year has increased enormously. Presently, RP is performed a six different hospitals in Denmark. No previous studies have meticulously described outcomes of RP in Denmark. This PhD-thesis focuses on surgical and oncological outcome after RP at Rigshospitalet. The primary purpose was to describe biochemical outcome, risk factors associated with positive surgical margins, and the impact of margin location on risk of biochemical recurrence.

MATERIAL AND METHODS:

The PhD-thesis is based on results from approximately 1300 men who underwent RP between 1995 and 2011 at Rigshospitalet. The patients have been followed prospectively in a local database. BR was defined as the first PSA ≥ 0.2 ng/ml and time to BR was calculated from the date of surgery. Analysis of time to BR was done using Kaplan-Meier estimation and Cox regression analysis including both pre- and postoperative parameters. The association between preoperative and surgical parameters, including surgeon and nerve-sparing surgery, and PSM was analysed using logistic regression analysis.

RESULTS:

The 10-year estimated BRFS was 75%, 60% and 39% for low-, intermediate-, and high-risk patients, respectively. An in-depth analysis of high-risk patients demonstrated a 10-year metastasis-

free and cancer-specific survival of 85% and 90%, respectively. A PSM was demonstrated to increase the risk of BR up to 3 fold. The location of PSM was found to be associated with the risk of BR, i.e. non-apical PSM had the highest risk of BR compared to margin negative and apical PSM, especially in pT2 tumours. A number of factors were found to correlate with the risk of PSM, especially preoperative PSA, surgeon and nerve-sparing surgery.

CONCLUSIONS:

This thesis demonstrates that outcome of RP at Rigshospitalet is comparable to international results. Our studies confirm the prognostic importance of PSM, also in pT2 disease, and indicate that location of PSM in pT2 may influence future selection of patients for adjuvant treatment. Further, the selection of candidates for nerve-sparing surgery seems to be associated with an increased risk of PSM and subsequent BR. Therefore, the selection for nerve-sparing surgery remains unclear.

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