On cribriform prostate cancer

C.F. Kweldam

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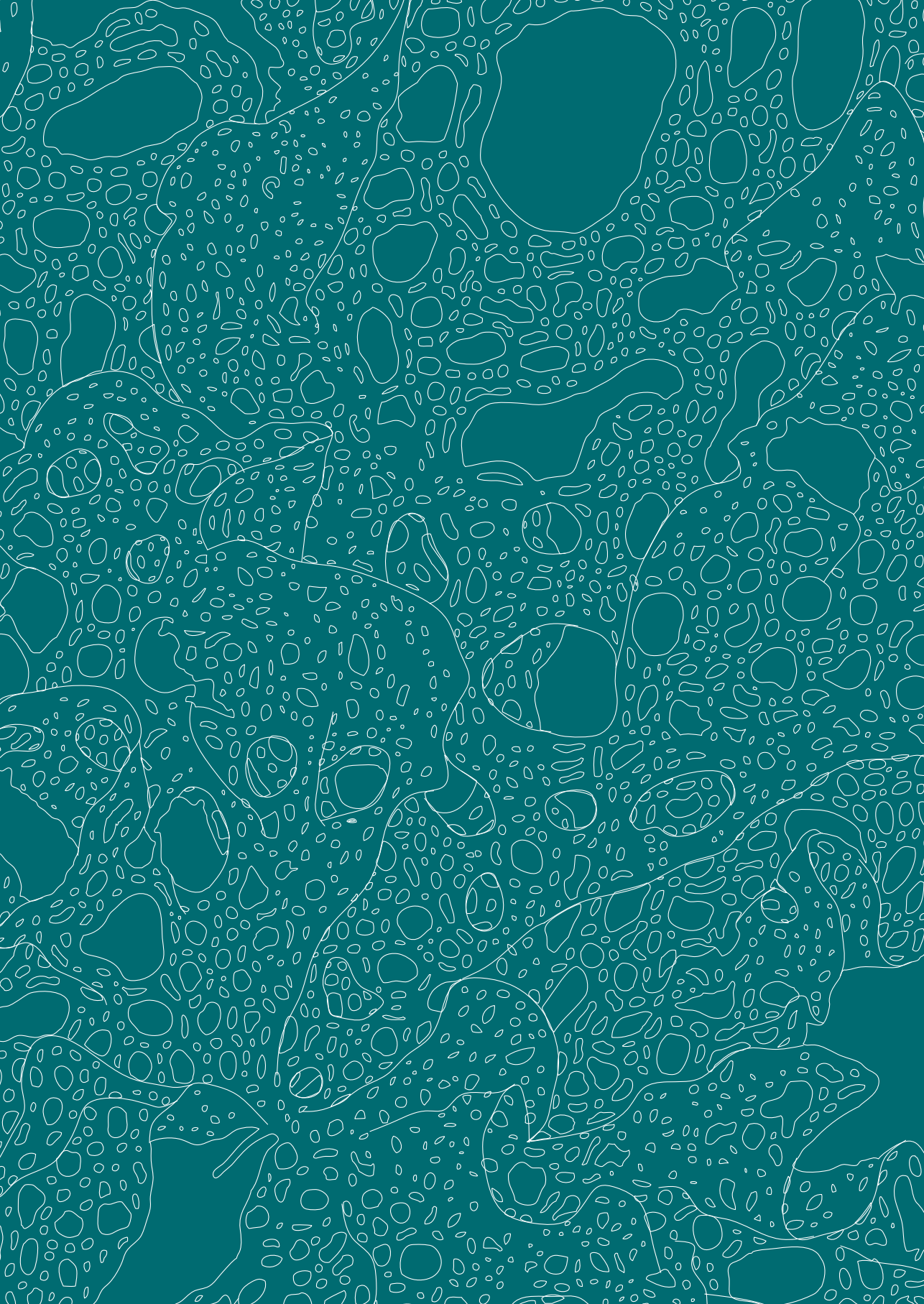

GENERAL INTRODUCTION

What is prostate cancer?

The prostate is a gland that is located below the urinary bladder and in front of the rectum. The normal prostate contains two main types of tissue: glandular tissue and fibromuscular stroma. The glands are lined by two cell layers: a flat basal cell layer and an overlying epithelial cell layer. The latter is responsible for producing a secretion that is added to the semen. The fibrous part of the stroma provides strength to the tissue, while the muscular part permits the prostate to contract and expel fluids. The prostate can be divided into several different anatomic regions, most important of which are the peripheral zone (outer part) and transition zone (inner part).

Hyperplasia, defined by an increase in the number of cells in a tissue or organ, mostly arises in the transition zone. Microscopically it is characterized by a well-circumscribed nodular proliferation of benign stromal and glandular elements. The hyperplastic glands are lined by two layers, i.e. basal cell layer and epithelial cell layer. Because benign prostatic hyperplasia involves the inner part of the prostate, the nodules often compress the urethra leading to lower urinary tract obstruction. Symptoms include difficulty in starting to urinate and intermittent interruption of the urinary stream while voiding. Benign prostatic hyperplasia is extremely common and occurs in almost all men as they age. About half of all men older than 75 years have symptoms related to hyperplasia.

Prostate cancer arises in the outer (peripheral) glands. Microscopically, the glands are usually smaller than benign glands and are lined by a single layer of epithelium. Prostate cancer lacks the basal cell layer seen in benign glands. In contrast to benign glands, malignant glands are more crowded and are able to invade the surrounding normal prostate tissue. Although most prostate cancers are small and asymptomatic, they may be palpable as irregular hard nodules on digital rectal examination. More advanced prostate cancers may present with symptoms, such as problems with urinating or blood in the urine or semen. In general, prostate cancers are discovered on the basis of an elevated serum prostate-specific antigen (PSA) level in the blood. A prostate biopsy is required to confirm the diagnosis of prostate cancer in each patient. Using a transrectal ultrasound (TRUS) the urologist inserts a thin, hollow needle through the wall of the rectum into the prostate to remove small tissue cylinders from the prostate. Most urologists take about 10-12 biopsies from different parts of the prostate. The samples are sent to a pathology laboratory and processed by technicians. The tissue will be embedded in a paraffin block and from each block one representative section of 3-5 µm is cut using a microtome. This section is mounted on a microscopic slide and stained with hematoxylin and eosin. After staining, the sections

are covered with a glass coverslip and evaluated by a pathologist under the microscope. The pathologist will assign a diagnosis and write a report. In the case of prostate cancer, the pathologist also assigns a grade, known as a Gleason score. The extent of a prostate cancer plays an important role in choosing treatments options for a patient and in predicting clinical outcome (prognosis). It is based on prostate biopsy Gleason score, serum PSA level at the time of diagnosis and results of any other tests that were done to find out how far the cancer has spread, e.g. bone scan, computed tomography (CT) scan or magnetic resonance imaging (MRI) scan. About 1 in 7 men will be diagnosed with prostate cancer during his lifetime, most of which are diagnosed in men aged 65 or older. The average age at the time of diagnosis is 66. Although prostate cancer follows an aggressive disease course in a significant number of men, most men diagnosed with prostate cancer do not die from the disease. About 1 man in 39 will die of prostate cancer.[1]

Therapy

Surgery - a radical prostatectomy - is a common treatment for prostate cancer. The major potential side effects of surgery are urinary incontinence and erectile dysfunction. Another treatment option is radiation therapy, which may be used in several ways. Most common side effects of radiotherapy are urinary problems, bowel problems, fatigue, ejaculatory problems and skin irritation. It can be used as a primary treatment to treat low-grade cancers and be administered along with hormone therapy for cancers that have grown outside the prostate and into nearby tissues. Radiation therapy is also used if the cancer has not been removed completely or comes back in the area of the prostate after surgery. Lastly, it can be used in advanced prostate cancer to help prevent or relieve symptoms. Hormone therapy, also known as androgen deprivation therapy (ADT), has the goal to reduce androgen (male hormone) levels in the body. Androgens are known to stimulate cell growth prostate cancer. By depriving androgen blood levels the tumor may shrink or grow more slowly over time. However, other organs besides the prostate also use androgens. Hormone therapy can subsequently lead to a wide range of side effects, e.g lowered libido, erectile dysfunction, hot flashes, nausea, diarrhea, liver damage and loss of bone density. Hormone therapy alone does not, however, cure a patient from prostate cancer. Hormone therapy is often administered to patients in whom the cancer has spread too far to be cured by surgery or radiation. Hormone therapy can also be used in case the cancer remains or comes back after surgery or radiation therapy. If the prostate cancer has spread to distant organs (metastasized) and hormone therapy is not effective anymore, chemotherapy is a final treatment option, but will not cure the patient from prostate

cancer. Because prostate cancer often grows very slowly, a significant number of men do not need active treatment for their prostate cancer. Instead, urologists offer active surveillance in which men with prostate cancer are not treated for their disease. Active surveillance usually includes a doctor's visit with a PSA test and DRE every 6 months, depending on the protocol. Several active surveillance protocols exist nowadays, of which PRIAS (Prostate cancer Research International: Active Surveillance) was initiated at the Erasmus Medical Center in Rotterdam.[2]

Prostate cancer grading by the pathologist: past and present

In one of the first publications on prostate cancer, which appeared in the first decade of the 20th century, it was already noted that the microscopic appearance of prostate cancer varies greatly.[3] More than hundred years ago several histological growth patterns, such as acinar, scirrhous and solid, were recognized. In 1966, dr. Donald Gleason developed a histological classification of prostate cancer, which was solely based on its architectural pattern rather than cytological features (Figure 1a).[4] He distinguished 5 basic architectural patterns, numbered grade 1-5. Higher grades were considered to reflect more aggressive behavior.

Pattern 1. Very well differentiated small and closely packed glands forming a circumscribed tumor mass. The glands are of a uniform size and do not infiltrate adjacent benign prostatic glands or stroma. The cells are characterized by having pale cytoplasm, small and uniform nuclei and very few mitoses.

Pattern 2. Similar to pattern 1 but with less well circumscribed glands showing greater variation in both size and shape. It was also noted that within glands cells may be piled into more than one layer and that a mild degree of cribriform pattern may be present.

Pattern 3. This pattern shows a wide variation in morphology ranging from glands similar to those seen in pattern 2 but with diffuse stromal penetration of tiny glands or single cells, to cribriform glands showing greater variation than those classified in pattern 2. Cords or masses of cells showing some degree of glandular differentiation may also be present.

Pattern 4. Closely packed, large, pale polygonal cells that resemble clear cell renal cell carcinoma. These cells usually show some features of glandular differentiation and there is typically diffuse stromal infiltration.

Pattern 5. Undifferentiated carcinoma with little or no gland formation.

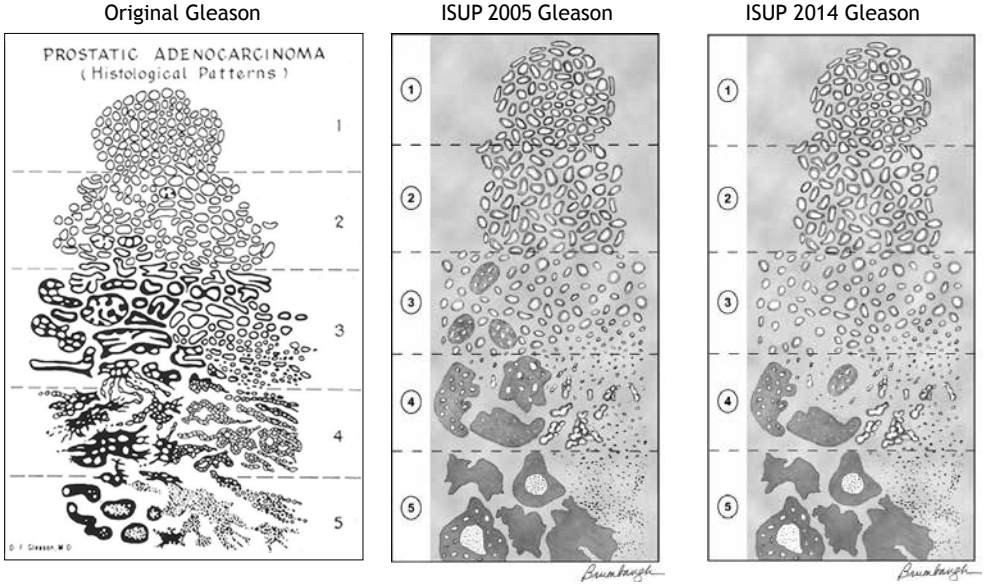

Figure 1. Gleason grading 1992 – present

Because the majority of the prostate cancers showed more than one type of growth pattern, he suggested assigning two patterns to each case in the order of predominance. This grading system of dr. Gleason was validated in 1974 and, after some modification of the definitions, has since then received a worldwide acceptance.[5] The Gleason score equals the sum of the two most common Gleason grades in radical prostatectomy, and, since 2005, the sum of the most common and highest Gleason grades in needle-biopsies.[6] To date, the Gleason grading system is one of the most powerful predictors of outcome in prostate cancer. The Gleason grading system has undergone a major modification in 2005 and an additional minor one in 2014 during International Society of Urological Pathologists (ISUP) consensus conferences (Figure 1).[6,7]

Gleason patterns 1 and 2 are for instance no longer in use in biopsies and the current Gleason score 6 (3+3) of 10 is the lowest possible score. Several growth patterns, which were originally considered Gleason grade 3, are now reassigned to a grade 4. In 2005 it was, for instance, agreed upon that large cribriform glands should be diagnosed as a Gleason grade 4, while small cribriform glands could still be assigned a Gleason grade 3. Because of the poor inter-observer reproducibility on diagnosing cribriform grade 3 glands, it was decided during the following ISUP consensus conference in 2014 that all cribriform glands should be considered as a Gleason grade 4 pattern. Grading the glomeruloid pattern has been controversial for many years. It has a typical morphology of a cribriform-like structure protruding into a gland attached to only one edge of the gland resulting in the structure

resembling a glomerulus of the kidney. Although based on scant scientific evidence, it was agreed upon in 2014 that glomeruloid structures are a Gleason grade 4 pattern. Originally, Gleason did not describe and specifically grade ill-formed glands. During the ISUP consensus conference in 2005, the ill-formed pattern was added to the Gleason grade 4 patterns as well. Consequently, from then on Gleason grade 3 only comprised well-delineated malignant glands. The contemporary Gleason grade 4 patterns are fused, ill-formed, cribriform and glomeruloid. Recently, the 5-tier prognostic grade grouping was introduced by the ISUP and recommended by the World Health Organization (WHO).[7] The grading system includes five distinct Grade Groups based on the modified Gleason score groups. Grade Group 1 = Gleason score ≤6, Grade Group 2 = Gleason score 3+4=7, Grade Group 3 = Gleason score 4+3=7, Grade Group 4 = Gleason score 8, Grade Group 5 = Gleason scores 9 and 10. Grade Grouping is not a novel grading system *per se*, but comprehensively distinguishes clinically significant patient cohorts.

The Gleason grade modification led to significant grade inflation.[8,9] One group, for instance, reported a significant decrease in Gleason score 6 (3+3) tumors from 48% to 22% of cases, while score 7 (3+4 and 4+3) tumors increased from 26% to 68%.[10] We believe that this relative increase is strongly associated with the inclusion of ill-formed glands as a Gleason grade 4 pattern since 2005. This pattern is, however, poorly reproducible among pathologists.[11-16] Reproducibility in recognizing Gleason pattern 4 prostate cancer on needle biopsy is most critical for clinical decision-making. In general, patients with Gleason score 6 on needle biopsy do not need immediate treatment and are often candidates for active surveillance. Patients with Gleason score 7 mostly undergo active treatment, i.e. surgery or radiotherapy. Today, both modified Gleason score 6 and 7 patients have a better prognosis than the historic ones, also known as the Will Rogers phenomenon.[9]

Cribriform prostate cancer

In 2011, Iczkowski et al. were the first to report that prostate cancer with cribriform growth has a worse biochemical-recurrence-free survival than those with "poorly formed glands".[17] Others and we have subsequently validated the adverse prognostic value of prostate cancers with a cribriform pattern using different patient groups and outcome measures, including biochemical recurrence, metastasis and disease-specific death.[18-22] Altogether these studies strongly suggest that cribriform growth in prostate cancer does not belong in the same risk group as, for instance, ill-formed glands. These studies had, however, been based on radical prostatectomy specimens. To affect clinical decision-making, it is essential to validate the prognostic value of cribriform growth in prostate cancer in pre-treatment needle biopsies.

Intraductal carcinoma of the prostate

In recent years the clinical significance of intraductal carcinoma of the prostate – a morphological mimicker of invasive cribriform carcinoma – has been acknowledged. The current concept is that it represents divergent differentiation of a common precursor that either spreads invasively or via pre-existing ducts.[23] Although not included in the Gleason grading system, intraductal carcinoma has been associated with Gleason grade 4 and 5 patterns, advanced tumor stage, biochemical recurrence and distant metastasis.[24-29] Invasive cribriform carcinoma and intraductal carcinoma are strictly speaking two different pathologic entities, but they morphologically mimic each other closely and it is possible they relate and exist on a pathological and biological continuum.[30,31] In fact, we believe that for many decades intraductal carcinoma has been diagnosed as a Gleason grade 4 or 5 pattern, as immunohistochemistry for basal cells was not available in the early days. Moreover, in line with the current 2014 ISUP recommendations, immunohistochemistry to distinguish invasive cancer carcinoma from intraductal carcinoma is not necessary. It should only be considered in cases where the results of the studies would change the case's overall grade, for example in cases lacking other Gleason grade 4 patterns.[7] Since the latter is extremely rare, it may indeed be more practical to regard them as one high-grade entity.

This thesis

This general aim of the thesis is to study the clinical relevance, interobserver reproducibility, and genetics of cribriform growth in prostate cancer. More specifically, the aims and outline of this thesis are

- To study the metastatic potential of modified Gleason score 3+3 prostate cancer in radical prostatectomies. (**Chapter 2**)

- To examine the prognostic value of individual Gleason grade 4 patterns in prostate cancer in radical prostatectomy and diagnostic biopsy specimens. (**Chapter 3 and 4**)

- To examine whether biopsy Gleason score 3+4 patients without cribriform growth could be candidates for active surveillance by comparing them with Gleason score 3+3 patients. (**Chapter 5**)

- To study the relation between Gleason grade 4 tumor percentage and cribriform prostate cancer in Gleason score 3+4 biopsies. (**Chapter 6**)

- To study the reproducibility of various Gleason grade 4 patterns, particularly that of cribriform growth, by undertaking an inter-observer reproducibility study among an international group of genitourinary pathologists. (**Chapter 7**)

- To study which genetic events are associated with cribriform growth in prostate cancer by using Next Generation Sequencing technology. (**Chapter 8**)

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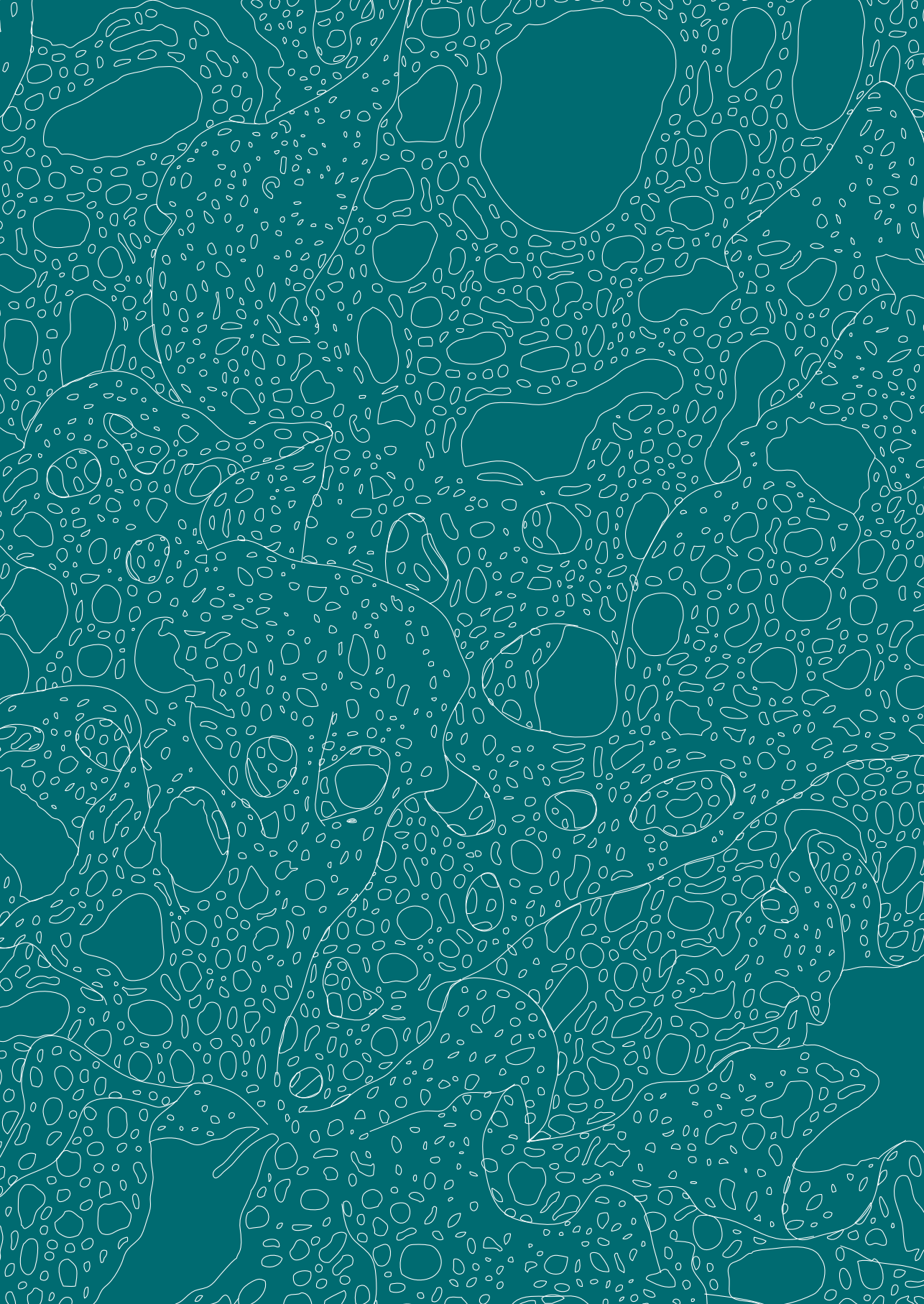

ABSTRACT

Background: To assess the metastasis-free survival (MFS) and disease-specific survival (DSS) in men with Gleason score ≤6 prostate cancer at radical prostatectomy (RP).

Patients and methods: We included 1101 consecutive RP patients operated between March 1985 to July 2013 at a single institution. The outcome variables were MFS and DSS. The post-operative survival was estimated by the Kaplan–Meier method.

Results: The Gleason score distribution of the study population (1101 patients) was Gleason score ≤6 (449, 41%), Gleason score 3+4=7 (436, 40%), Gleason score 4+3=7 (99, 9%) and Gleason score 8-10 (117, 11%). The median post-operative follow-up was 100 months (IQR 48-150). During follow-up 197 men (18%) died of whom 42 (3.8%) from prostate cancer-related causes. A total of 19/1101 patients (1.7%) had documented lymph node metastasis at time of operation: none with Gleason score ≤6, seven with Gleason score 3+4=7 (1.6%), six with Gleason score 4+3=7 (6.1%) and six with Gleason score 8-10 (5.1%). Distant metastasis occurred in 56/1101 patients (5.1%): none with Gleason score ≤6, 23 with Gleason score 3+4=7 (5.3%), 17 with Gleason score 4+3=7 (17%) and 16 with Gleason score 8-10 (14%). Disease-specific death, stratified per Gleason score group was: none in ≤6, 16 (3.7%) in 3+4=7, 16 (16%) in 4+3=7 and 10 (8.5%) in 8-10 group.

Conclusion: No metastasis or disease-specific death were seen in men with Gleason score ≤6 prostate cancer at RP, demonstrating the negligible potential to metastasize in this large subgroup patients with prostate cancer.

INTRODUCTION

The Gleason grading system is a strong predictor for disease progression in prostate cancer and one of the most important parameters for therapeutic clinical decision-making. For instance, many patients with Gleason score 6 on needle biopsy do not require immediate therapeutic intervention and are often eligible for active surveillance.[1] In contrast, patients with Gleason score ≥7 prostate cancer generally undergo active treatment for their disease. In most studies, clinical outcome of prostate cancer is measured by biochemical recurrence after radical prostatectomy (RP), which does not reflect tumor biology *per se*. In the present study, we assessed the biochemical recurrence-free survival (BCRFS), metastasis-free survival (MFS) and disease-specific survival (DSS) in a large cohort of men with Gleason score ≤6 at RP at a single institution.

PATIENTS AND METHODS

Patient population

Between March 1985 and July 2013, 1101 consecutive hormone naïve patients underwent RP for prostate cancer at the Erasmus Medical Center, Rotterdam, The Netherlands. RP specimens were routinely examined at the Department of Pathology of our institute. At pathological evaluation, Gleason score, extra-prostatic extension, seminal vesicle involvement, bladder neck invasion and surgical margin status were recorded for each patient. From 1985 to 2005, the classic Gleason grading system was applied, while modified Gleason grading was used from 2005.[2-4] At our institution, Gleason grade 4 was considered as a tertiary pattern and not included in the final Gleason score, if it encompassed <5% of the prostate cancer volume both before and after the introduction of modified Gleason grading. In 894 (81%) patients a pelvic lymph node dissection was performed at time of RP. In case intra-operative frozen sections demonstrated lymph node metastasis, RP was not performed; respective patients were not included in our study cohort. All pathologic slides were available for review.

Follow-up

After surgery, patients were monitored routinely at our outpatient clinic. Local recurrence was determined by a palpable mass or tissue biopsy in the presence of an elevated Prostate Specific Antigen (PSA) level. Biochemical recurrence was defined as a PSA level of ≥0.2 ng/mL, assessed at two consecutive time points at least 3 months after radical prostatectomy. Metastasis was defined as presence of prostate cancer in a lymph node or at a distant site with radiologic or pathologic confirmation. Outcome variables were BCRFS, defined as time after radical prostatectomy to biochemical recurrence; MFS, defined as time after radical prostatectomy to metastasis (lymph node, distant metastasis or both); DSS, defined as time after radical prostatectomy to death attributed to prostate cancer; overall survival defined as time after radical prostatectomy to all-cause death. Death and disease-specific death were verified by medical record review and death certificates. All relevant clinical, pathologic and follow-up data were recorded and regularly updated in a prospective study database (MW). Pathologic tumor (pT) stage was categorized according to the 2009 TNM system.[5]

Statistics

Continuous clinico-pathologic parameters (age at time of operation, follow-up and PSA level at time of diagnosis) of 4 Gleason score subgroups (≤6, 3+4=7, 4+3=7 and 8-10) were compared using the Independent-Samples Kruskal-Wallis Test. The Pearson's Chi-square (X^2) test was used for categorical parameters (pelvic lymph node dissection, pT-stage, and surgical margins). Survival probabilities were estimated by the Kaplan-Meier method. All statistics were performed using SPSS 21 (SPSS Inc., Chicago, USA). A two-sided $P<0.05$ was considered significant.

RESULTS

Baseline patient characteristics

The clinic-pathological characteristics and follow-up information of the 1101 selected patients are depicted in Table 1. The median (interquartile range, IQR) age at time of operation was 64 (60–68) years. The median (IQR) PSA level was 5.8 (3.9–9.1) ng/mL. The median (IQR) follow-up was 100 (48–150) months. During follow-up, 197 men (18%) died of whom 42 (3.8%) died from prostate cancer-related causes. In all, 19 (1.7%) and 56 (5.1%) patients had lymph node and distant metastasis, respectively.

Table 1. Clinico-pathologic and follow-up information of prostate cancer patients treated by radical prostatectomy (n=1101).

Clinico-pathologic parameter		Mean (median; IQR) or n (%)
Age (years)		63 (64; 60-68)
PSA level (ng/mL)		8.4 (5.8; 3.9-9.1)
Follow-up after radical prostatectomy (months)		100 (100; 48-150)
Gleason score	≤6	449 (41)
	7	535 (49)
	3+4	436 (40)
	4+3	99 (9.0)
	8-10	117 (11)
Pelvic lymph node dissection		894 (81)
pT-stage (TNM 2009)	T2	664 (60)
	T3a	351 (32)
	T3b	86 (7.8)
Positive surgical margins		333 (30)
Biochemical recurrence		258 (23)
Local recurrence		52 (4.7)
Lymph node metastasis		19 (1.7)
Distant metastasis		56 (5.1)
Lymph node and/or distant metastasis		70 (6.4)
Overall death		197 (18)
Disease-specific death		42 (3.8)

Gleason score in relation to clinical outcome

Initial statistical analysis of the Gleason score ≤6 group, revealed that six patients had developed metastasis and five patients died from prostate cancer during follow-up. These patients were operated between 1988 and 2001, and graded according to the classic Gleason score system. All slides from respective cases were retrieved from our archives and reviewed by a urogenital pathologist (G.v.L). At review, all prostate cancer specimens revealed Gleason grade 4 growth patterns in >5% of the tumor volume, and were re-assigned a modified Gleason score 7 (Fig. 1, Table 2). The predominant Gleason grade 4 growth pattern at revision was the formation of cribriform glands.

Table 2. Histopathological review of cases with classic Gleason score ≤6 at original diagnosis with metastasis, disease-specific death or both.

Patient #	Lymph node metastasis	Distant metastasis	Disease-specific death	Reviewed Gleason score	Undergraded pattern(s)
1		Yes	Yes	4+3=7	Cribriform
2			Yes	3+4=7	Cribriform, fused, intraductal carcinoma
3			Yes	3+4=7	Cribriform, fused
4		Yes		3+4=7	Cribriform, fused
5		Yes	Yes	3+4=7	Fused, glomeruloid
6	Yes			3+4=7	Cribriform, fused, ill-defined, intraductal carcinoma
7		Yes		3+4=7	Fused, ill-defined, tertiary Gleason grade 5
8		Yes	Yes	3+4=7	Cribriform, fused, glomeruloid

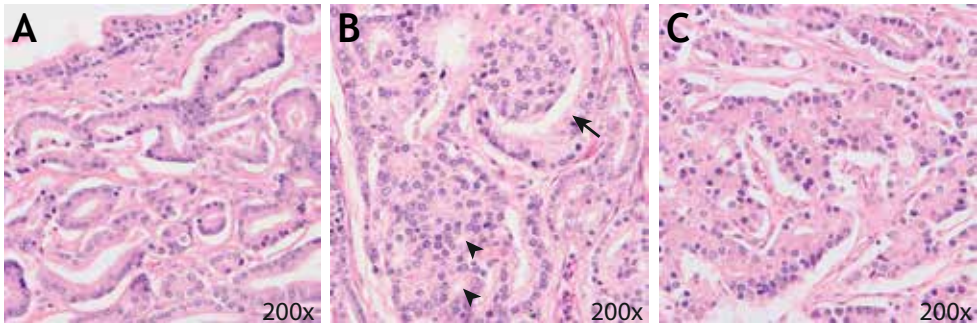

Figure 1. Prostate adenocarcinoma originally graded as Gleason score 3+3=6, with under-recognized Gleason grade 4 patterns with A) ill-defined, B) combined glomeruloid (arrow)/ cribriform (arrowheads) and C) fused growth patterns.

After review, the final distribution of the Gleason score in the study population was as follows: Gleason score ≤6 (449, 41%), Gleason score 3 + 4 = 7 (436, 40%), Gleason score 4+3=7 (99, 9%) and Gleason score 8–10 (117, 11%). The distribution within the Gleason score 8–10 group was 3+5=8 (37, 31%), 4+4=8 (20, 17%), 4+5=9 (30, 26%), 5+3=8 (15, 13%), 5+4=9 (14, 12%) and 5+5=10 (one, 1%). The clinico-pathological and follow-up information of patients with prostate cancer treated by RP, stratified by Gleason score (≤6, 3+4=7, 4+3= 7 and 8–10) are summarized in Table 3. Metastasis and disease-specific death occurred only in patients with prostate cancer with Gleason score ≥3+4=7. In Fig. 2, the BCRFS, MFS, DSS and overall survival are depicted for all Gleason-score subgroups. None of the 449 patients with Gleason score ≤6 prostate cancer with a median (IQR) follow-up of 120 (77–160) months developed metastasis or died from prostate cancer-related causes.

Table 3. Clinico-pathologic and follow-up information of prostate cancer patients treated by radical prostatectomy, stratified by Gleason score (n=1101).

Clinico-pathologic parameter		Mean (median; IQR) or n (%)				P value
		GS ≤6 (n=449)	GS 3+4=7 (n=436)	GS 4+3=7 (n=99)	GS 8-10 (n=117)	
Age (years)		64 (63; 60-67)	65 (64;59-68)	66 (66; 61-70)	67 (66; 62-69)	<0.001 †
PSA level (ng/mL)		6.2 (4.8; 3.4-7.2)	9.4 (6.2; 4.0-9.3)	11 (8.8; 5.6-14)	14 (9.7; 6.6-15)	<0.001 †
Follow-up after radical prostatectomy (months)		120 (120;77-160)	94 (87; 39-150)	97 (95; 19-140)	64 (50; 12-98)	<0.001 †
Pelvic lymph node dissection		396 (88)	329 (76)	82 (83)	87 (74)	<0.001 ‡
pT-stage (TNM 2009)	T2	369 (82)	231 (53)	39 (39)	25 (21)	<0.001 ‡
	T3a	78 (17)	117 (41)	41 (41)	55 (47)	
	T3b	2 (0.4)	28 (6.4)	19 (19)	37 (32)	
Positive surgical margins		90 (20)	276 (37)	31 (32)	53 (45)	<0.001 ‡
Biochemical recurrence		49 (11)	113 (26)	45 (46)	51 (44)	
Local recurrence		10 (2.2)	22 (5.0)	13 (13)	7 (6.0)	
Lymph node metastasis		0	7 (1.6)	6 (6.1)	6 (5.1)	
Distant metastasis		0	23 (5.3)	17 (17)	16 (14)	
Overall death		66 (15)	85 (20)	31 (31)	15 (13)	
Disease-specific death		0	16 (3.7)	16 (16)	10 (8.5)	

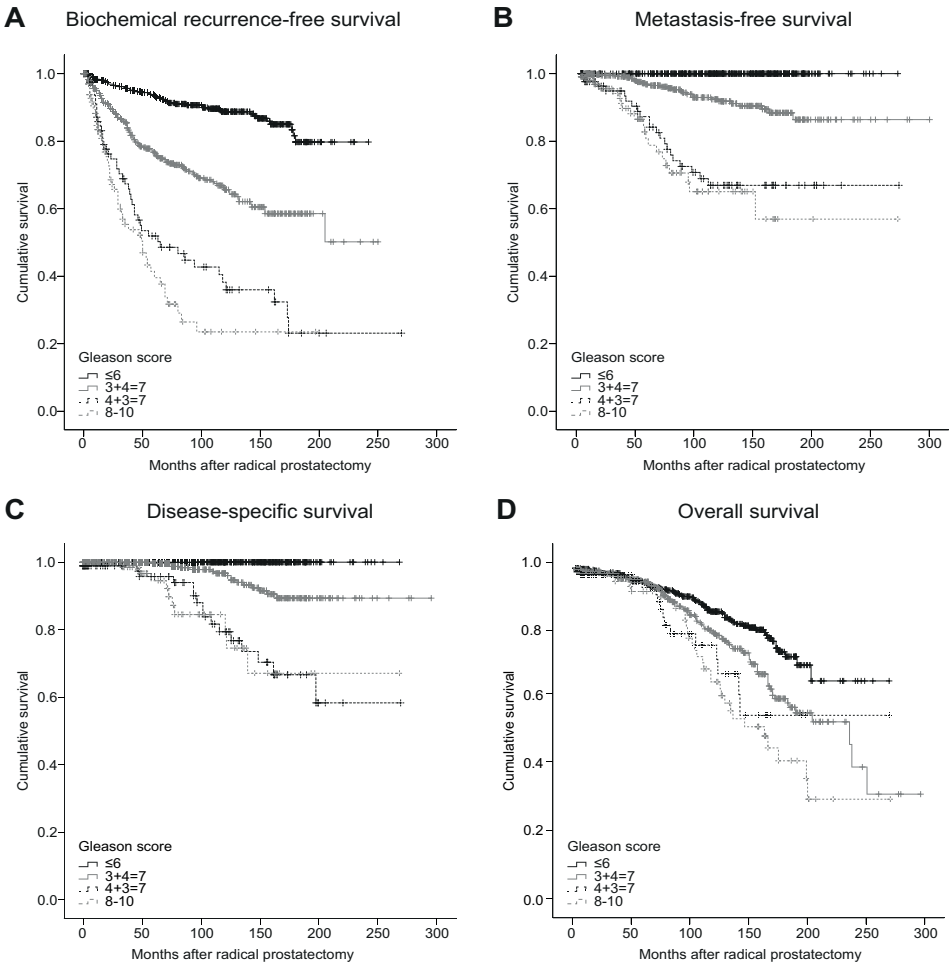

Figure 2. Kaplan-Meier estimates of A) biochemical recurrence-free survival, B) metastasis-free survival, C) disease-specific survival and D) overall survival.

Clinico-pathologic characteristics of Gleason score ≤6

In all, 82% (369/449) of patients with Gleason score ≤6 prostate cancer at RP had organ-confined disease (pT2); 78 men (17%) had extra-prostatic extension (pT3a) and two (0.4%) had seminal vesicle involvement (pT3b). To validate whether Gleason score ≤6 prostate cancer had the potential to spread into extra-prostatic tissues, we randomly reviewed 30 RPs with Gleason score ≤6 and ≥pT3. In all 30 cases the pT stage and Gleason score were concordant with initial pathological findings.

Surgical margins were positive in 90 RP specimens (20%). In 396/449 patients (88%), a pelvic lymph node dissection was performed, in which no lymph node metastasis was observed. Biochemical recurrence was observed in 49 patients (11%). In all, 22 of these 49 patients (45%) had positive surgical margins and 25 men had ≥pT3 (51%). Local recurrence was seen in 10 patients (2%), two of whom (20%) had positive surgical margins and six ≥pT3 (60%).

DISCUSSION

Gleason grading is one of the most important parameters for clinical decision-making and prediction of disease outcome. In pathological practice, a Gleason grade is purely based on the assignment of architectural prostate cancer growth patterns. The Gleason score is determined by adding the two most common Gleason grades in RP specimens; in needle biopsies, the most common and highest Gleason grades are added. Essentially, Gleason grade pattern 1–3 encompass well-delineated malignant glands; the presence of cribriform, fused, ill-defined and glomeruloid glands are not acceptable for Gleason grade 3 prostate cancer.[2,6,7]

The metastatic potential of Gleason score ≤6 prostate cancer is a topic of interest, as previous studies have demonstrated negligible rates of biochemical recurrence after radical prostatectomy and salvation radiotherapy.[8-10] In addition, Hernandez *et al*. have shown that patients with organ-confined Gleason score ≤6 prostate cancer do not develop post-operative metastases nor die from prostate cancer.[10] Recent analysis of >14 000 RPs performed at Johns Hopkins Medical Institutions demonstrated that lymph node metastasis does not occur in men with modified Gleason score ≤6 prostate cancer during follow-up.[11] Our present study is consistent with these findings, and additionally shows that distant metastasis and disease-specific death do not occur in non-organ confined Gleason score ≤6 prostate cancer as well.

Eggener *et al*. previously reported on disease-specific death in a large cohort of 12,000 RPs.[12] In their study, the 15-year disease-specific mortality rates in patients with classic and modified Gleason score ≤6 were 0.2-1.2%. In the other Gleason score groups the 15-year disease-specific mortality rates were 4.2-6.5% in Gleason score 3+4=7, 6.6-11% in Gleason score 4+3=7 and 26-37% in Gleason score 8-10. The latter rates are consistent with our present findings, except for the Gleason score 8–10 group. In our present cohort, 10 of 117 patients with Gleason score 8–10 (8%) died from prostate cancer with a median follow-up of 66 months, which is lower than the death rate in the Gleason score 4+3=7 group. The low number of metastases and disease-specific deaths in the Gleason score 8–10 group could be explained by a selection bias, as RP is generally not the first choice of therapy in men with high Gleason score at needle-biopsy in our institute. Furthermore, it might be due to the fact that our institute had a policy up to 2002 not to perform RP when intraoperative frozen sections showed lymph node metastasis. Also, the relative amount of high-grade prostate cancer, i.e. Gleason grade 4 or 5 is less in Gleason score 3+5=8 than in Gleason score 4+3=7 tumors, which might also explain the worse outcome of Gleason score 4+3=7 patients.[13] In our present study, nearly one-third of the Gleason score 8–10 patients had

Gleason score 3+5=8. Finally, the relatively short follow-up in the Gleason score 8–10 group [median (IQR) 50 (12–98) months] could have led to an underestimation of metastasis or death attributed to prostate cancer in this subgroup of patients.

That Gleason score ≤6 prostate cancer has very low, if any, potential to metastasize raises the question whether Gleason score ≤6 prostate cancer should be considered as a malignant tumor at all. Berman *et al*. discussed several problems of diagnosing Gleason 6 as cancer vs. benign disease.[14] First, most prostate cancers occur in older men, progress slowly and are not life threatening. Therefore it is unlikely that a man with the lowest score on RP, Gleason score 6, will die from prostate cancer. Although up to 90% of patients with prostate cancer undergo RP, only half of them have potentially life-threatening cancer (Gleason score ≥7).[15]

One of the most important arguments against diagnosing Gleason score 6 as a benign tumor is under-sampling of high-grade cancer on prostate needle biopsy. Unlike many tumors, prostate cancer is a very heterogeneous disease, and susceptible to sampling error. For instance, in a recent and large study containing 7643 RPs with corresponding needle biopsies, Epstein *et al*. reported that 36% of cases (1841/ 5071) were upgraded from a needle-biopsy Gleason score 6 to a higher grade at RP.[16] Based on large active surveillance studies in men with Gleason score 6 on biopsy, up to 33% of the patients still need therapeutic intervention primarily due to Gleason score upgrading.[15,17-21] Furthermore, in our present study 17% of Gleason score 6 prostate cancer had extraprostatic expansion (pT3a) and 0.4% seminal vesicle involvement, indicating that these tumors can show aggressive behavior locally.

The major limitation of our present study was that not all RP specimens were pathologically reviewed and scored according to the modified Gleason score. Recently, Dong *et al*. re-graded 806 radical prostatectomies with Gleason score 3+3=6 and Gleason score 3+4=7 prostate cancer according to the modified Gleason grading system.[22] They found an upgrade of 34% from classical Gleason score 3+3=6 prostate cancer to modified Gleason score 7 or 8 at radical prostatectomy. However, not a single case of Gleason score ≥7 was downgraded to a Gleason score ≤6 at RP.

Therefore, we assume that pathological review in our present study would have reduced the number of patients with Gleason score ≤6 at RP, but would not have changed our finding that Gleason score ≤6 prostate cancer does not metastasize or lead to disease-specific death. Another imitation of our present study was the unavailability of data on the cause of death in 28 patients (3%), of whom 12 had Gleason score ≤6 at RP. In addition, not all patients with Gleason score ≤6 underwent a lymph node dissection at the time of RP, so that their metastatic status remains unknown. Also, it cannot be excluded that patients who

did not undergo RP because of intraoperative lymph node metastasis actually had Gleason score ≤6 prostate cancer at RP. The present Gleason score ≤6 group had the longest follow-up in this cohort (median 120 months), but longer follow-up may be needed to further exclude long-term metastatic potential of Gleason score ≤6 at RP.

The significant emergence of metastatic potential of prostate cancer, when Gleason grade 4 or 5 patterns are observed, has major implications for understanding the biology of prostate cancer. As growth pattern seems to be associated so strongly with disease outcome, it is intriguing to understand the cellular mechanisms that underlie various growth patterns. Review of prostate cancer initially diagnosed as Gleason score ≤6 with progression, revealed the presence of cribriform growth in most cases. This pattern was also most frequently seen in the revised aggressive Gleason score ≤6 prostate cancer in the series of Ross *et al*.[11] Recently Dong *et al*. reported that cribriform growth pattern, in particular, was an independent predictor for biochemical recurrence as well as metastasis after RP, suggesting that Gleason grade 4 architectural patterns could provide important prognostic information beyond the current Gleason classification system.[23]

CONCLUSION

No metastasis or disease-specific death were seen in men with Gleason score ≤6 prostate cancer at RP, demonstrating the negligible potential to metastasize in this large subgroup of patients with prostate cancer.

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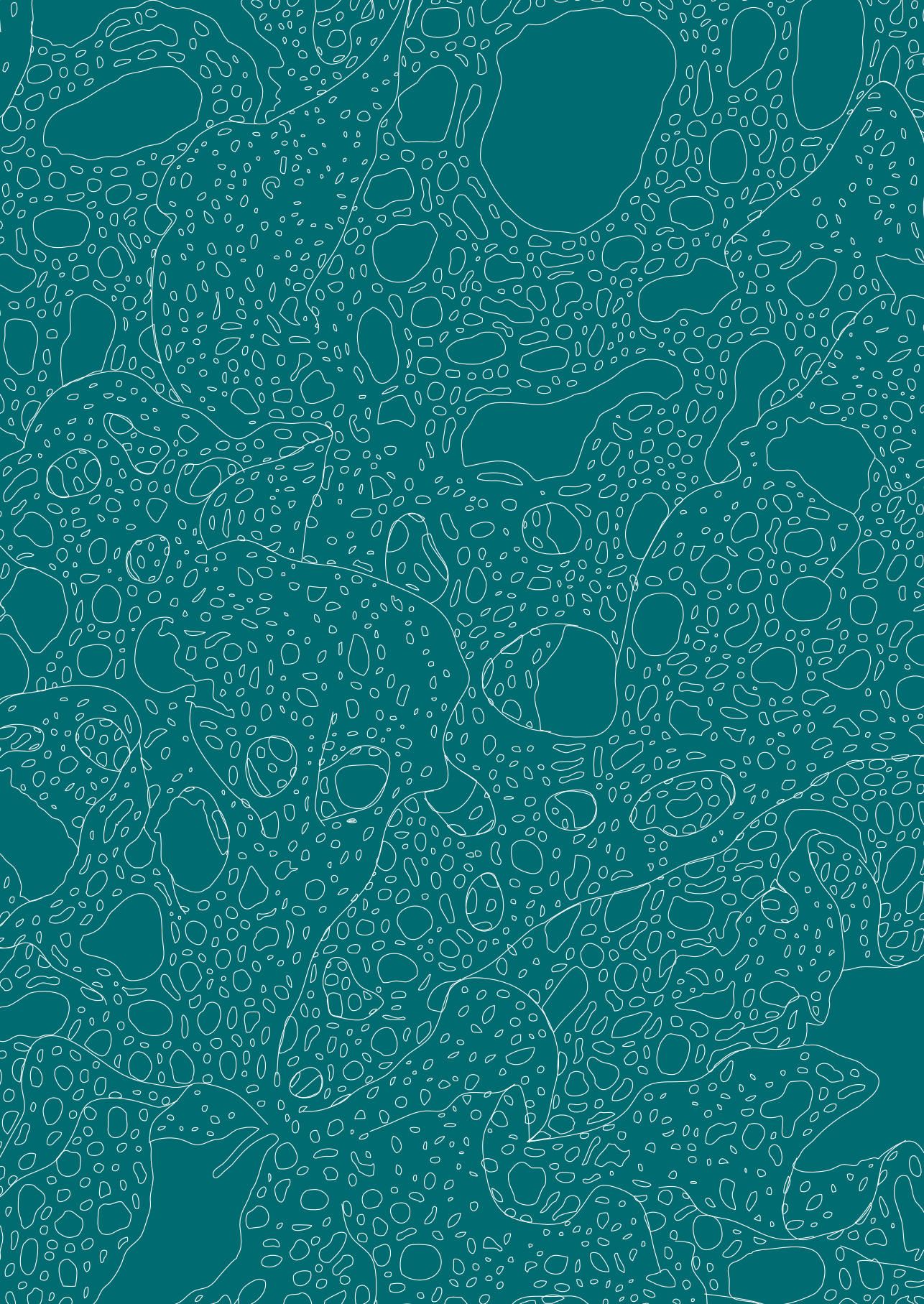

ABSTRACT

Background: Patients with Gleason score 7 prostate cancer on radical prostatectomy demonstrate a wide range in clinical outcome. Gleason grade 4 prostate cancer encompasses a heterogeneous group of tumor growth patterns including fused, ill-defined, cribriform and glomeruloid glandular structures. Our objective was to determine the prognostic value of different Gleason grade 4 growth patterns.

Patients and methods: We performed a nested case-control study among 535 patients with Gleason score 7 prostate cancer at radical prostatectomy, treated between March 1985 and July 2013 at a university hospital in The Netherlands. We analyzed 52 cases (with metastasis, disease-specific mortality or both) and 109 controls, matched for age, PSA level and pT stage. Presence of the following Gleason grade 4 patterns was recorded: fused, ill-defined, cribriform and glomeruloid. Intraductal carcinoma of the prostate and tertiary Gleason grade 5 were additionally assessed. Outcomes were metastasis-free survival and disease-specific survival. We used Cox proportional hazards regression to determine the predictive value of Gleason grade 4 patterns for survival time.

Results: The overall prevalence of Gleason grade 4 patterns was as follows: fused 75% (n=121), ill-defined 64% (n=102), cribriform 48% (n=83) and glomeruloid 25% (n=40). Cribriform pattern was the only pattern with an unequal distribution between cases and controls. Forty-two out of 52 cases (81%) had cribriform growth pattern versus 41/109 controls (38%). In multivariate analysis, presence of cribriform growth was an adverse independent predictor for distant metastasis-free survival (HR 8.0, 95% CI 3.0-21; P<0.001) and disease-specific survival (HR 5.4, 95%CI 2.0-15, P=0.001).

Conclusion: cribriform growth in Gleason grade 4 is a strong prognostic marker for distant metastasis and disease-specific death in patients with Gleason score 7 prostate cancer at radical prostatectomy.

INTRODUCTION

The widely used Gleason grading system for prostate cancer discerns 5 different grades based on the architectural tumor growth pattern.[1] The Gleason score is determined by adding the two most common Gleason grades in radical prostatectomies; in needle-biopsies the most common and highest Gleason grades are added. The Gleason grading system is an important predictor of disease progression, and one of the most important variables for clinical decision-making. In 2005, large cribriform and ill-defined glands, classically described as Gleason grade 3, were redefined as Gleason grade 4.[2] Later small cribriform and glomeruloid glands have been reconsidered Gleason grade 4 as well.[3,4] This grade migration has led to a decline in reporting of Gleason score 6 on radical prostatectomy, joined by a relative increase of Gleason score 7 prostate cancer.[5] Whereas patients with modified Gleason score 6 on radical prostatectomy represent a group with excellent outcome, patients with Gleason score 7 demonstrate a wide range in clinical outcome.[6-8] Risk stratification within the Gleason score 7 patient population remains a challenge, and additional prognostic factors are needed. The objective of this study was to determine the predictive value of distinctive Gleason grade 4 growth patterns for metastasis and disease-specific death in men with Gleason score 7 prostate cancer on radical prostatectomy.

MATERIALS AND METHODS

Study design

We identified 535 hormone-naïve patients with Gleason score 7 prostate cancer on radical prostatectomy, treated between March 1985 and July 2013 at Erasmus MC, Rotterdam, The Netherlands. In our cohort 56 patients had documented metastasis or disease-specific death during follow-up ('cases'). The control group consisted of 112 Gleason score 7 patients without documented metastasis or disease-specific death. We matched the control group for the following 3 variables: age at time of surgery, serum Prostate Specific Antigen (PSA) level at time of diagnosis (ng/mL) and pT stage.[9] We randomly selected controls in the pT2 and pT3a group with follow-up ≥120 months. Limits for age were ≥47 and ≤74 years. Limits for PSA level were ≥0 and ≤100 ng/mL. In 7 patients, histopathologic slides and blocks could not be retrieved from the archive (4 cases and 3 controls), leaving 52 cases and 109 controls for analysis with all slides and clinico-pathologic information available.

Pathologic evaluation

After operation, all radical prostatectomy specimens were routinely examined at the Department of Pathology of our institute. At pathologic evaluation, Gleason score, pT stage and surgical margin status were recorded for each patient. From 1985 to 2005, the classic Gleason grading system was applied; the modified Gleason grading was used after 2005. The 2009 TNM classification was used to assess pT stage.[9] A positive surgical margin was defined as extension of the tumor into the inked surface of the specimen.

The investigator (CK) and a board certified pathologist with expertise in urogenital pathology (GvL), reviewed all slides and routinely determined the modified Gleason score.[2] Both reviewers were blinded to the patients' outcome. The presence of Gleason grade 4 growth patterns was specifically recorded in each specimen. In addition, we assessed the presence of tertiary Gleason grade 5 and intraductal carcinoma of the prostate in each specimen, since both have been associated with adverse clinical outcome.[10-14] The following Gleason grade 4 growth patterns, as defined by the ISUP-modified Gleason grading scheme, were scored as follows: 1) fused glands included fused well- and poorly formed glands (Fig. 1A). 2) Ill-defined glands comprised glands with poorly formed or absent glandular lumina (Fig. 1B). Only a cluster of such glands was acceptable, to exclude the possibility of tangentially sectioned Gleason pattern 3 glands. 3) Cribriform was characterized by a glandular proliferation with multiple punched-out lumina, without intervening stroma (Fig. 1C). 4) Glomeruloid glands were defined as the presence of dilated glands containing a cribriform proliferation that is attached to only one edge of the gland, resulting in the

structure resembling a glomerulus (Fig. 1D). Tertiary Gleason grade 5 was defined as presence of 1) solid sheets, cords, or single cells with no glandular differentiation or 2) comedocarcinoma with central necrosis surrounded by papillary, cribriform, or solid masses in less than 5% of the radical prostatectomy specimen. Intraductal carcinoma was defined as a well-circumscribed lesion surrounded by an intact basal cell layer distended by overtly malignant-appearing epithelial populations (Fig. 1E).[15] Clear distention of prostate glands and presence of necrosis were used as cut-offs to distinguish intraductal carcinoma from high-grade prostatic epithelial neoplasia. To distinguish small foci of cribriform pattern from fused glands we applied two criteria. Contact of the majority of tumor cells with adjacent stroma and more linear orientation of lumina instead of rounded lumina were both in favor for fused glands. In addition, we did not use a size threshold for cribriform fields. When cribriform and intraductal carcinoma could not be distinguished morphologically, immunohistochemistry for basal cells (34BE12) was performed (n=6); presence of basal cells was considered supportive of intraductal carcinoma (Fig. 1F). Consensus was reached in all cases during a joint session.

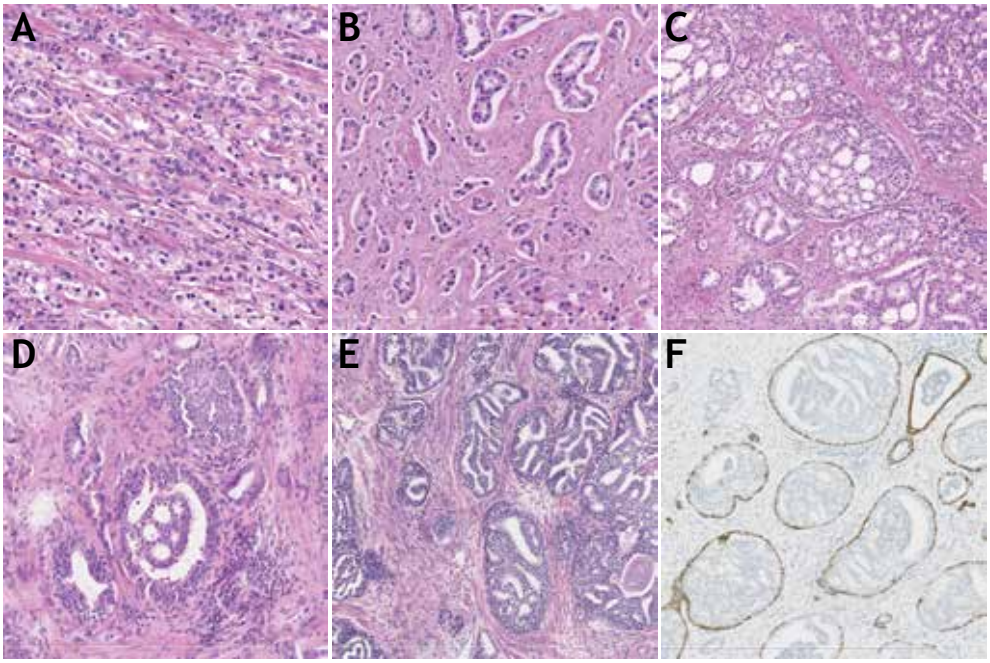

Figure 1. Gleason grade 4 patterns and intraductal carcinoma. A, fused glands. B, ill-defined glands. C, cribriform glands. D, glomeruloid gland. E, intraductal carcinoma. F, 34BE12 immunohistochemistry, demonstrating the presence of basal cells supportive for intraductal carcinoma.

Follow-up

After surgery, patients were monitored annually at our outpatient clinic. Biochemical recurrence was defined as a PSA level of ≥0.2 ng/mL, assessed at two consecutive time points >3 months apart after radical prostatectomy. Metastasis was defined as presence of prostate cancer in a lymph node or at a distant site, with radiologic or pathologic confirmation. Since all lymph node metastases in this cohort were diagnosed at time of operation (follow-up 0 months), they were not included as an endpoint but as a covariate in multivariate analysis of distant metastasis and disease-specific death. Distant metastases in this study were all hematogenous. Outcome variables were biochemical recurrence-free survival defined as time after radical prostatectomy to biochemical recurrence; metastasis-free survival defined as time after radical prostatectomy to distant metastasis; disease-specific survival defined as time after radical prostatectomy to death attributed to prostate cancer; overall survival defined as time after radical prostatectomy to all-cause death. Death and disease-specific death were administered by medical record review and death certificates. All relevant clinical, pathologic and follow-up data were recorded and regularly updated in a prospective study database (MW).

Statistics

Continuous clinico-pathologic variables were analyzed using the Mann-Whitney U test, and categorical variables using the Pearson's Chi-square (X^2) test. Correlation coefficients were calculated by the Spearman's rank Correlation test. Survival probabilities were estimated by the Kaplan-Meier method. Unadjusted two-group comparisons for survival time were made with log-rank testing. We used Cox proportional hazards regression to determine the predictive value of Gleason grade 4 patterns for survival time. Age, PSA level, Gleason score, pT stage, surgical margin status, lymph node status, Gleason grade 4 patterns, intraductal carcinoma and tertiary Gleason grade 5 were all included in the multivariable analysis as potential confounders. Dummy variables were created to convert pT stage into series of binary groups. All statistics were performed using SPSS 22 (SPSS Inc., Chicago, USA). A two-sided P value <0.05 was considered significant.

RESULTS

Patient characteristics

The clinico-pathologic characteristics are listed in Table 1. The median follow-up in controls was 160 months (IQR 120-190), which was significantly longer than the follow-up of the cases (100 months; IQR 78-150; P=0.001). As expected from the matching, cases and controls had a similar distribution for age, PSA level and pT stage (Table 1). Gleason score 4+3=7 was more frequent in cases than controls (48% vs. 19%, X^2 P=0.001). Furthermore, cribriform pattern and intraductal carcinoma were both more often present in cases than controls: 81% vs. 38% (X^2 P<0.001) and 58% vs. 33% (X^2 P=0.003) respectively. Cases and controls showed a similar distribution for fused, ill-defined and glomeruloid Gleason grade 4 patterns, tertiary Gleason grade 5, number of Gleason grade 4 patterns, and surgical margin status.

Biochemical recurrence occurred in 44/52 (85%) of the cases and in 45/109 (41%) of the controls. In cases, the median time to biochemical recurrence was 19 months (IQR 11-37); in controls this was 61 months (IQR 28-110) (log rank P<0.001). A total of 45 (87%) cases had metastatic disease, of which 11 were lymph node metastasis (21%) all discovered at time of operation, and 37 distant metastasis (71%). The median time to distant metastasis was 67 months (IQR 46-94). Thirty men (60%) of the cases died from prostate cancer. The median time to disease-specific death was 110 months (IQR 91-140). The overall mortality rate was 70% in cases, and 14% in controls.

Table 1. Clinico-pathologic characteristics of the entire study population (N=535), cases (n=52) and controls (n=109).

Clinico-pathologic parameter		Entire cohort (N=535)	Cases (n=52)	Controls (n=109)	P value*
			Mean (median; IQR) or n (%)		
Age at time of surgery (years)		64 (65; 60-68)	63 (63; 60-68)	63 (59-68)	0.76 †
PSA level (ng/mL)		9.8 (6.4; 4.2-10)	12 (7.8; 5.3-13)	12 (7.4; 5.4-16)	0.60 †
Follow-up after radical prostatectomy (months)		94 (91; 37-150)	110 (100; 78-150)	140 (160; 120-190)	0.001 †
Gleason score	3+4	436 (81)	27 (52)	88 (81)	0.001 ‡
	4+3	99 (19)	25 (48)	21 (19)	
pT-stage (2009)	T2	270 (50)	10 (19)	22 (20)	0.48 ‡
	T3a	218 (41)	25 (48)	61 (56)	
	T3b	47 (8.8)	17 (33)	26 (24)	
Cribriform growth pattern			42 (81)	41 (38)	<0.001 ‡
Fused			38 (73)	83 (76)	0.67 ‡
Ill-defined			30 (58)	72 (66)	0.30 ‡
Glomeruloid			9 (17)	31 (28)	0.13 ‡
Number of Gleason grade 4 patterns	1		31 (24)	4 (13)	0.16 †
	2		58 (45)	17 (53)	
	3		33 (26)	9 (28)	
	4		7 (5.4)	2 (6.2)	
Intraductal carcinoma			30 (58)	36 (33)	0.003 ‡
Tertiary Gleason grade 5			5 (9.6)	8 (7.3)	0.62 ‡

* The P value was based on comparison between cases and controls. † Mann-Whitney U test. ‡ Pearson's Chi-square (X^2) test.

Cribriform growth pattern is associated with Gleason score 4+3=7 and intraductal carcinoma

The clinico-pathologic characteristics of the study population stratified by either the presence or absence of cribriform pattern are listed in Table 2. Presence of cribriform pattern was 3.0 times more frequent in patients with Gleason score 4+3=7 (42% vs. 14% in absence of cribriform pattern, X^2 P<0.001). Intraductal carcinoma was seen in 54/83 patients with cribriform pattern (65%), and vice versa cribriform pattern in 54/66 (82%) patients with intraductal carcinoma (Spearman's ρ=0.51, P<0.001), while intraductal carcinoma without cribriform pattern occurred in 12/78 patients (15%). Additionally, cribriform pattern was positively associated with presence of multiple Gleason grade 4 patterns (Spearman's ρ=0.54, P<0.001). Also, lymph node metastases were more prevalent in specimens with cribriform pattern (11% vs. 2.6% in absence of cribriform pattern, X^2 P=0.037). By contrast, ill-defined glands were more prevalent when cribriform pattern was absent (76% vs. 52%; X^2 P=0.002).

Table 2. Clinico-pathologic characteristics of the study population (n=161) stratified by cribriform growth pattern.

		Cribriform growth pattern		
		Present (n=83)	Absent (n=78)	
Clinico-pathologic parameter		Mean (median; IQR) or n (%)		P value
Age at time of surgery (years)		63 (63; 59-68)	63 (64; 60-68)	0.30 †
PSA level (ng/mL)		14 (8.1; 5.4-17)	9.1 (7.1; 5.2-12)	0.15 †
Follow-up after radical prostatectomy (months)		120 (120; 73-170)	140 (150; 120-180)	0.012 †
Gleason Score	3+4=7	48 (58)	67 (86)	<0.001 ‡
	4+3=7	35 (42)	11 (14)	
pT-stage (2009)	T2	14 (17)	18 (23)	0.33 ‡
	T3a	43 (52)	43 (55)	
	T3b	26 (31)	17 (22)	
Positive surgical margin		35 (43)	45 (46)	0.78 ‡
Fused		66 (80)	55 (71)	0.19 ‡
Ill-defined		43 (52)	59 (76)	0.002 ‡
Glomeruloid		22 (27)	18 (23)	0.62 ‡
Number of Gleason grade 4 patterns	1	6 (7.2)	29 (37)	<0.001 †
	2	32 (39)	43 (55)	
	3	36 (43)	6 (7.7)	
	4	9 (11)	0 (0.0)	
Intraductal carcinoma		54 (65)	12 (15)	<0.001 †
Tertiary Gleason grade 5		11 (13)	2 (2.6)	0.013 ‡

† Mann-Whitney U test. ‡ Pearson's Chi-square (X^2) test.

Predictors for biochemical recurrence: seminal vesicle invasion, positive surgical margins and cribriform pattern

When cribriform pattern was present, the median time to biochemical recurrence was 34 months (IQR 11-88), and 120 months (IQR 40-170) when cribriform pattern was absent (log rank P<0.001) (Figure 2A). Age, PSA level, Gleason score 4+3=7, seminal vesicle

invasion (pT3b), positive surgical margins, and intraductal carcinoma were significant predictors for biochemical recurrence-free survival in a univariate analysis (data not shown). In multivariable analysis the following 3 variables were independent predictors for biochemical recurrence-free survival: seminal vesicle invasion (HR 2.6, 95% CI 1.2-5.7, P=0.014), positive surgical margins (HR 1.9, 95% CI 1.2-3.0, P=0.010) and cribriform pattern (HR 2.0, 95% CI 1.2-3.4, P=0.006).

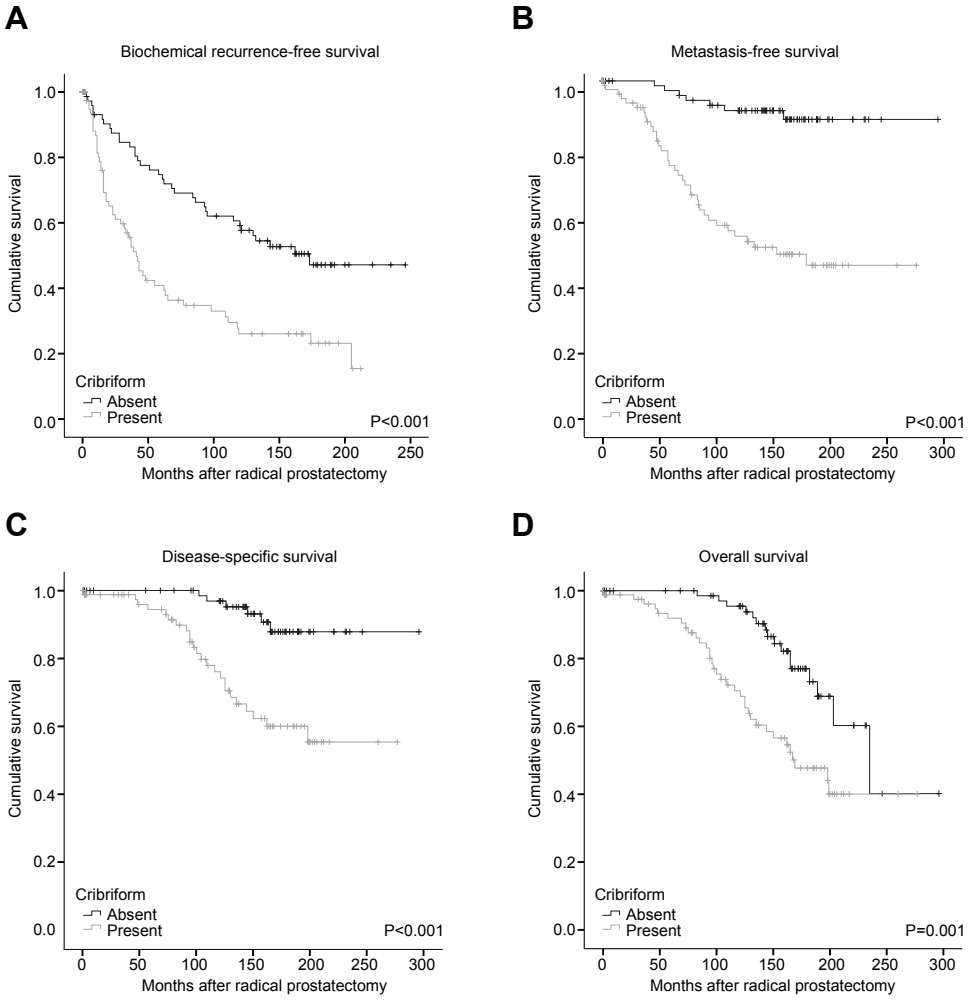

Figure 2. Kaplan-Meier estimates on impact of cribriform growth pattern in A, biochemical recurrence-free survival. B, distant metastasis-free survival. C, disease-specific survival. D, overall survival.

Cribriform pattern is a strong predictor for distant metastasis and disease-specific death

The median time to distant metastasis in patients with cribriform pattern was 88 months (IQR 42-160), and 150 months in men without cribriform pattern (IQR 120-180) (log rank P<0.001) (Figure 2B). Crude and adjusted hazard ratios for distant metastasis are listed in Table 3. Univariate predictors for distant metastasis were Gleason score 4+3=7, cribriform pattern, intraductal carcinoma and tertiary Gleason grade 5; in multivariable analysis cribriform pattern was the only independent predictor for distant metastasis (HR 8.0, 95% CI 3.0-21, P<0.001), adjusted for age, PSA level, Gleason score, pT stage, surgical margin status, lymph node status, Gleason grade 4 patterns, intraductal carcinoma and tertiary Gleason grade 5. In contrast, fused pattern was associated with a decreased adjusted hazard ratio for distant metastasis (HR 0.47, 95% CI 0.22-1.0, P=0.048). The median time to disease-specific death in men with cribriform pattern was 120 months (IQR 76-170), and 150 months (IQR 120-180) in men without cribriform pattern (log rank P<0.001) (Figure 2C). Crude and adjusted hazard ratios for disease-specific survival are listed in Table 3. Univariate predictors for disease-specific survival were Gleason score 4+3=7, cribriform pattern and intraductal carcinoma (Table 3). Independent predictors for disease-specific survival were both Gleason score 4+3=7 (HR 3.1, 95% CI 1.4-7.1, P=0.007) and cribriform pattern (HR 5.4, 95%CI 2.0-15, P=0.001). Furthermore, the overall survival in patients with cribriform pattern was shorter than in patients without cribriform pattern (log rank P=0.001) (Figure 2D).

Cribriform pattern in Gleason score 3+4=7 and Gleason score 4+3=7 subpopulations

Primary Gleason grade 4 (Gleason score 4+3=7) was unequally distributed between cases and controls (Table 1). Although Cox regression analysis compensated for this unequal distribution, we additionally studied the predictive value of cribriform pattern separately in Gleason score 3+4=7 (n=115) and Gleason score 4+3=7 (n=46) subpopulations. In Gleason score 3+4=7, cribriform pattern was an independent predictor for both distant metastasis-free survival (20 events) (HR 6.0, 95% CI 2.0-18, P=0.001) (log rank P<0.001) and disease-specific survival (13 events) (HR 4.9, 95% CI 1.3-18, P=0.017) (log rank P=0.013) in multivariable analysis. Due to the limited number of events (n=12) and number of covariates no models could be fitted for distant metastasis-free survival in Gleason score 4+3=7 prostate cancer patients. Cribriform pattern was, however, an independent predictor for disease-specific survival (12 events) in Gleason score 4+3=7 (HR 17, 95% CI 2.2-130, P=0.006).

DISCUSSION

The clinical outcome of Gleason score 7 prostate cancer after radical prostatectomy is highly variable. Pathologically, Gleason grade 4 prostate cancer encompasses a heterogeneous group of growth patterns, defined as fused, ill-defined, cribriform and glomeruloid. Our objective was to determine the prognostic value of individual Gleason grade 4 patterns in Gleason score 7 prostate cancer patients.

In this study, we found that presence of cribriform pattern in radical prostatectomy specimens was a major predictive factor for distant metastasis and disease-specific death of prostate cancer. In fact, cribriform pattern was the strongest predictor for both adverse clinical events in multivariate analysis, adjusted not only for established clinico-pathologic variables (age, PSA, Gleason score, pT stage and surgical margins), but also for contemporary additional pathologic variables such as intraductal carcinoma and tertiary Gleason grade 5 pattern. Therefore, identification of cribriform growth in daily pathology practice is a new, fast and cheap adjunct to predict adverse clinical outcome.

The clinical significance of Gleason grade 4 patterns has only since recently become an area of interest. Dong *et al*. found that metastasis occurred 5 times more frequently in patients with cribriform pattern at radical prostatectomy (13% vs. 2.6% when cribriform pattern absent) in a consecutive series of 241 patients.[16] These results are well in line with the findings of the current study. However, Dong *et al*. did not adjust for other important variables, such as lymph node status, intraductal carcinoma and tertiary Gleason grade 5.

A strong point of this study is the use of metastasis-free and disease-specific survival as endpoints since they objectively reflect aggressive tumor biology, while biochemical and local recurrence also depend on surgical margin status, and do not necessarily indicate metastatic tumor potential. In addition, we included contemporary pathologic characteristics such as intraductal carcinoma and tertiary Gleason grade 5, as covariates in our analysis. It is striking that presence of cribriform growth either as invasive prostate cancer or as intraductal expansion is associated with disease outcome. In both cribriform pattern and intraductal carcinoma, a majority of tumor cells has lost physical contact with surrounding stromal matrix or basement membrane, respectively, and only connect to adjacent epithelial tumor cells. The presence of intraductal carcinoma has been associated with high-grade cancer and adverse outcome.[10,12,13] In our study, presence of cribriform pattern was significantly associated with intraductal carcinoma. Two hypotheses of intraductal carcinoma evolution have been proposed.[17] First, established Gleason grade 4 or 5 prostate cancer could infiltrate and expand pre-existing glands; this theory is supported by the fact that intraductal carcinoma is rarely found in absence of high-grade prostate cancer.

Alternatively, high-grade prostatic intraepithelial neoplasia, which is generally accepted as prostate cancer precursor, might evolve into intraductal carcinoma and consecutively invasive high-grade prostate cancer. The molecular and biological connections of separate intraductal and invasive growth patterns are intriguing and clinically important, but still poorly understood.

A limitation of the current study is the nested case-control design, in which the cases had Gleason score 4+3=7 prostate cancer more often than the controls. Although Cox regression analysis compensated for the unequal Gleason score distribution, we performed additional analysis in 3+4 and 4+3 prostate cancer subgroups, which confirmed the prognostic value of cribriform pattern. Another caveat of our study was the strict delimitation of Gleason grade 4 patterns in 4 groups. This subdivision reflects the categories defined by the ISUP/ WHO, but does not take into account subtle architectural variations such as small and large cribriform fields, locally fused glands and complex fused structures with intervening stroma. In this study, we did not determine the relative percentage or volume of Gleason grade 4. While subgroup analysis of cribriform growth in Gleason score 3+4=7 (<50% Gleason grade 4) and 4+3=7 (≥50% Gleason grade 4) revealed independent prognostic value in both groups, further delineating of Gleason grade 4 percentage might have influenced outcome. Interestingly, Ross *et al*. suggested in their large study of lymph node metastasis that even the slightest presence of cribriform growth could give rise to metastasis.[7] Furthermore, we did not take prostate cancer volume or an associated parameter into account. Although prostate cancer volume might be a confounder in our study, our group has previously shown that it did not add prognostic value to established pathologic variables.[18] We matched for pT stage as a surrogate of prostate cancer volume in this study.

The outcome of our study is of significant clinical relevance. Currently, patients with Gleason score 3+4=7 are considered to be at low to intermediate risk in clinical practice; they even might be candidates for active surveillance. However, based on our results, Gleason score 7 patients with cribriform growth are more likely to be candidates for treatment. If validated on diagnostic prostate needle-biopsies, cribriform growth might influence therapeutic decision-making in clinical practice.

CONCLUSION

Cribriform growth is a novel and strong independent histopathological predictor for distant metastasis-free survival and disease-specific survival in patients with Gleason score 7 prostate cancer at radical prostatectomy.

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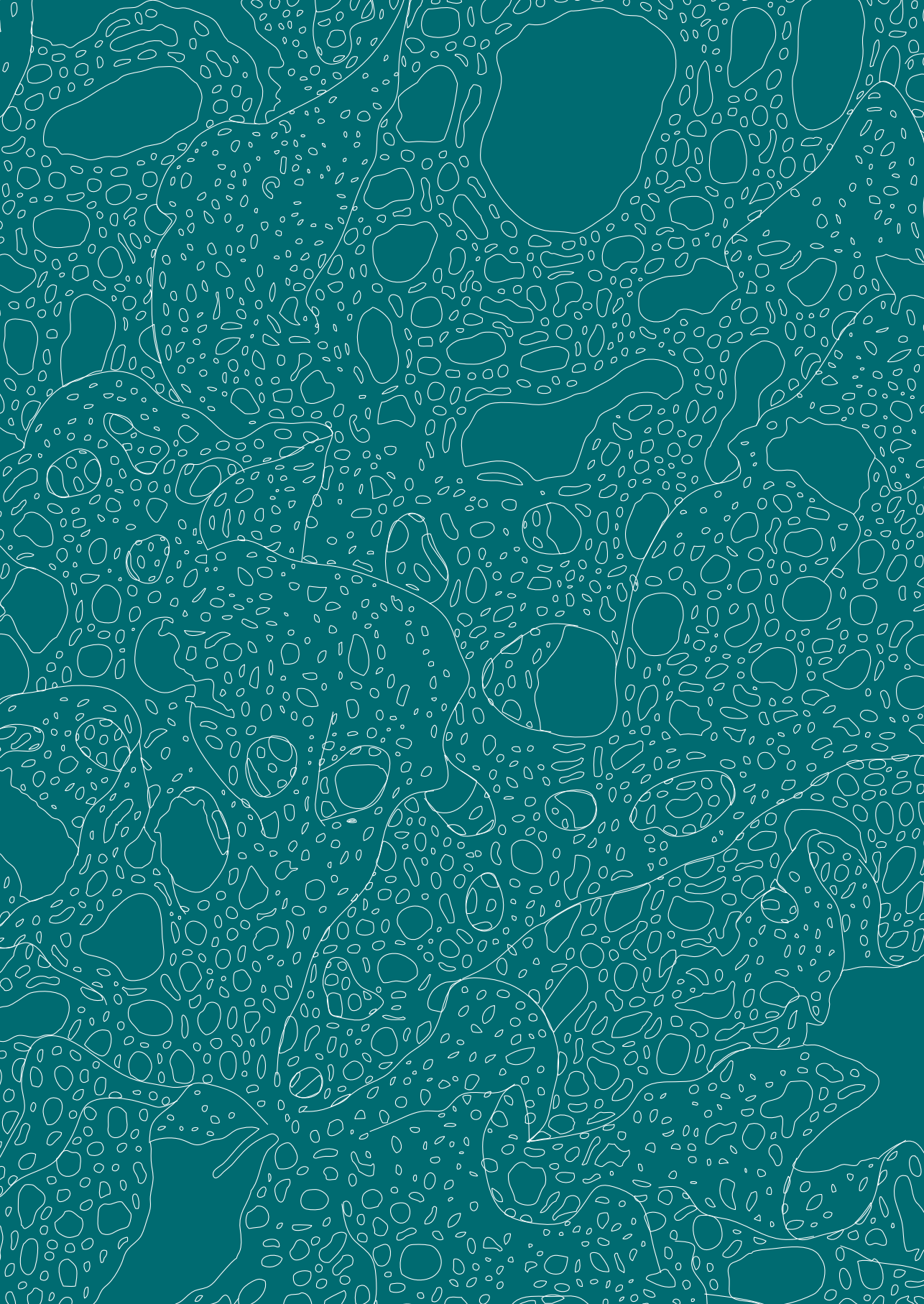

ABSTRACT

Background: Invasive cribriform and intraductal carcinoma in radical prostatectomy specimens have been associated with an adverse clinical outcome. Our objective was to determine the prognostic value of invasive cribriform and intraductal carcinoma in pre-treatment biopsies on time to disease-specific death.

Patient and methods: We pathologically revised the diagnostic biopsies of 1031 patients from the first screening round of the European Randomized Study of Screening for Prostate Cancer 1993-2000). Ninety percent of all patients (n=923) had received active treatment, whereas 10% (n=108) had been followed by watchful waiting. The median follow-up was 13 years. Patients who either had invasive cribriform growth pattern or intraductal carcinoma were categorized as CR/IDC+. The outcome was disease-specific survival. Relationships with outcome were analyzed using multivariable Cox regression and log-rank analysis.

Results: In total, 486 patients had Gleason score 6 (47%) and 545 had ≥7 (53%). The 15-year disease-specific survival rates were 99% in Gleason score 6 (n=486), 94% in CR/IDC- Gleason score ≥7 (n=356) and 67% in CR/IDC+ Gleason score ≥7 (n=189). CR/IDC- Gleason score 3+4=7 patients did not have statistically different survival probabilities from those with Gleason score 6 (P=.30), while CR/IDC+ Gleason score 3+4=7 patients did (P<.001). In multivariable analysis CR/IDC+ status was independently associated with a poorer disease-specific survival (HR 2.6, 95% CI 1.4-4.8, P=.002).

Conclusion: We conclude that CR/IDC+ status in prostate cancer biopsies is associated with a worse disease-specific survival. Active surveillance criteria may potentially be extended to CR/IDC- Gleason score 3+4=7 prostate cancer, as these patients have similar survival rates to those with Gleason score 6.

INTRODUCTION

The management of newly diagnosed prostate cancer is challenging because of its heterogeneity in histology, genetics and clinical outcome. Today, clinical-decision making mostly depends upon serum Prostate Specific Antigen (PSA) level, clinical tumor stage, and pathologic biopsy Gleason score – a grading system based on architectural tumor patterns. While patients with the lowest Gleason scores ≤6 have an excellent outcome, those with the highest Gleason scores (9-10) have the worst.[1]

The clinical outcome of Gleason score 7 prostate cancer patients is highly variable. Improving risk assessment is of particular interest, as Gleason score 7 prostate cancer on biopsy is an important clinical threshold for active treatment. Recent studies have suggested that the broad contemporary definition of the Gleason grade 4 pattern may be one of the explanations for the variable outcomes of patients with Gleason score 7 prostate cancer.[2-5] Architecturally, four Gleason grade 4 growth patterns are recognized: ill-formed, fused, glomeruloid and cribriform.[1,6] Recently, cribriform pattern has been associated with adverse outcome after radical prostatectomy in Gleason score 7 prostate cancer.[2-5]

In recent years the clinical relevance of intraductal carcinoma of the prostate – a high-risk lesion defined as malignant epithelium filling large acini or ducts with preservation of basal cells – has been acknowledged. Although not included in the Gleason score, intraductal carcinoma has been associated with high Gleason scores, advanced tumor stage, biochemical relapse and distant metastasis.[7-12] Intraductal carcinoma can, however, microscopically mimic invasive cribriform carcinoma requiring additional immunohistochemistry for their distinction. Studies on the prognostic value of invasive cribriform and intraductal carcinoma have mostly been based on radical prostatectomy specimens.[2-5,11,13] The aim of this study was to determine the prognostic value of invasive cribriform and intraductal carcinoma in diagnostic biopsies on time to disease-specific death.

MATERIALS AND METHODS

Patient selection

We included all 1078 men from the first screening round of the Dutch part of the European Randomized Study of Screening for Prostate Cancer (ERSPC), who had been diagnosed with prostate cancer between November 1993 and March 2000 in Erasmus Medical Center, Rotterdam, The Netherlands. The trial protocol has been published previously.[14,15] The ERSPC is an ongoing multicenter randomized screening trial that was initiated in the early 1990s to evaluate the effect of screening with PSA testing on disease-specific mortality rates. Exclusion criteria of the present study were unavailability of slides or paraffin blocks for review (n=24), and presence of lymph node or distant metastasis at time of diagnosis (n=23), leaving 1031 patients for analysis.

Pathological evaluation

Three investigators (C.K., I.K., G.v.L.), who were blinded to patient information and outcome, revised all histopathological slides. For each biopsy core we recorded tumor percentage, tumor length (mm), Gleason score, presence of intraductal carcinoma, and presence of Gleason grade 4 and 5 growth patterns.[1] The overall tumor percentage per patient was defined as the sum of total tumor length (mm) divided by the sum of total biopsy length (mm). The label CR/IDC+ was given to patients who either had invasive cribriform carcinoma, intraductal carcinoma or both, CR/IDC− to those who had neither. CR/IDC specific tumor percentage per patient was defined as the sum of total length CR/IDC glands (mm) divided by the sum of total biopsy length (mm). Gleason grading was performed according to the 2014 ISUP recommendations.[1] To distinguish invasive cribriform carcinoma from intraductal carcinoma, and high-grade prostatic epithelial neoplasia (HGPIN) from intraductal carcinoma we used morphological criteria as described by Guo et al.[7] In case morphological distinction between invasive cribriform carcinoma and intraductal carcinoma was not certain (105/193, 54%), we applied high-molecular-weight-keratin immunohistochemistry to detect the presence of basal cells.

Clinical follow-up

After diagnosis and initial treatment, patients were semi-annually monitored by chart review to assess potential progression and secondary treatments. The cause of death was evaluated by an independent cause-of-death committee, where death due to causes related to screening were also counted as prostate cancer deaths.[16] Although data on the occurrence of distant metastases were available, we did not include this endpoint in our study, as these events largely overlapped with the number of disease-specific deaths.

Statistical analysis

Continuous parameters were analyzed by the Mann-Whitney U test or Kruskal-Wallis test, categorical parameters by the Pearson's Chi-square (x^2) test. Non-normally distributed continuous variables underwent log base 2 transformation such that effects related to a doubling in unit. We estimated survival probabilities using the Kaplan-Meier method. Unadjusted comparisons for survival time were made using log-rank tests with censoring of men lost to follow-up or dying of other causes. Crude and adjusted hazard ratios (HRs) for survival time were calculated using Cox proportional hazards regression. The concordance index (c-index) was used to quantify the ability of single variables and combinations of variables in multivariable models to discriminate between patients with and without the event of interest.[17] The c-index takes values between 0.5 and 1, where 0.5 indicates that the model is not better than chance classification and 1 means perfect discrimination.[18] Regression models were compared using the Likelihood-ratio test. All statistical analyses were performed in R version 3.1.2 (R, Vienna, Austria). Two-sided P values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics

The median age of the entire cohort (N=1031) was 66 years (IQR 62-71) and the median follow-up 13 years (IQR 9.4-16, Table 1). In total, 90% of all patients (n=923) had received active treatment, whereas 10% (n=108) had been followed by watchful waiting. A total of 496 patients died during follow-up, 72 of whom from prostate cancer. The majority (53%) of patients had Gleason score 3+4=7 or higher. Gleason score was positively associated with age, PSA level, tumor percentage and percentage of positive cores. The most frequently observed Gleason grade 4 pattern in Gleason score 3+4=7 or higher was ill-formed (80%), followed by fused (53%), cribriform (20%) and glomeruloid (15%). Presence of cribriform growth was the most discriminative Gleason grade 4 pattern between Gleason score 3+4=7 and 4+3=7 (7.7% versus 37%, x^2 P<.001). We found a similar association for intraductal carcinoma (13% versus 42%, x^2 P<.001). Intraductal carcinoma co-existed with invasive cribriform carcinoma in 57 out of 111 patients (51%). Invasive cribriform and intraductal carcinoma were predominantly seen in Gleason score 4+3=7 and higher prostate cancer. In total, 193 patients had CR/IDC+ status; the distribution among Gleason score is shown in Table 1. Most low-risk patients had undergone radical prostatectomy whereas high-risk patients had received radiotherapy.

Prognostic value of CR/IDC status

Presence of intraductal carcinoma (crude HR 7.6, 95% CI 4.8-12, P<.001, c-index=0.697) and invasive cribriform carcinoma (crude HR 6.3, 95% CI 3.9-10, P<.001, c-index=0.639) were both significantly associated with worse disease-specific survival in univariate analyses. The combined CR/IDC+ status was also strongly associated with worse disease-specific survival (crude HR 11, 95% CI 6.6-18, P<.001, c-index=0.758) and was similar if intraductal carcinoma and invasive cribriform carcinoma were analyzed as separate predictors in a model (c-index = 0.761). When separating each Gleason score group for CR/IDC status the disease-specific survival rates were significantly lower in CR/IDC+ patients with Gleason score 3+4=7, 8, and 9-10. (Figure 1). Although we saw some evidence of lower survival rates in CR/IDC+ Gleason score 4+3=7, differences between groups did not meet conventional levels of statistical significance (log rank P=.054). The Gleason score 6 group contained only 4 CR/IDC+ patients (all intraductal carcinoma). CR/IDC- Gleason score 3+4=7 patients did not have significantly different survival probabilities from those with Gleason score 6 (log rank P=.30), while CR/IDC+ Gleason score 3+4=7 patients had significantly worse survival rates than those with Gleason score 6 (log rank P<.001) and CR/IDC- Gleason score

Table 1. Patient and tumor characteristics (N=1031)

	Gleason score 6 (n=486)	Gleason score 3+4=7 (n=310)	Gleason score 4+3=7 (n=104)	Gleason score 8 (n=64)	Gleason score 9-10 (n=67)	P value
	Mean (median, IQR) or n(%)					
Age at diagnosis (years)	66 (66, 61-70)	66 (67, 62-71)	68 (69, 65-71)	68 (69, 66-72)	67 (67, 64-71)	<.001 ‡
PSA level at diagnosis (ng/mL)	5.8 (4.7, 3.5-6.9)	8.8 (5.8, 4.0-9.0)	15 (8.6, 4.7-18)	19 (11, 6.2-17)	16 (9.4, 5.4-16)	<.001 ‡
Percentage of positive cores (%)	31 (29, 17-43)	2.9 (3.0, 2.0-4.0)	50 (43, 29-71)	55 (50, 40-71)	62 (57, 43-86)	<.001 ‡
Tumour percentage (%)	24 (17, 9.5-33)	43 (44, 27-57)	51 (51, 33-68)	51 (52, 33-66)	56 (56, 41-74)	<.001 ‡
Gleason grade 4 patterns						
Ill-formed		227 (73)	63 (85)	51 (80)	64 (96)	<.001 †
Fused		153 (49)	46 (62)	32 (50)	39 (58)	.07 †
Cribriform		24 (7.7)	38 (37)	23 (36)	26 (39)	<.001 †
Glomeruloid		33 (11)	14 (19)	13 (20)	11 (16)	.02 †
Gleason grade 5 patterns						
Single cells and strands				35 (55)	61 (91)	<.001 †
Solid				3 (4.7)	16 (24)	.002 †
Intraductal carcinoma	4 (0.82)	41 (13)	44 (42)	18 (28)	32 (48)	<.001 †
CR/IDC+ status	4 (0.82)	54 (17)	60 (58)	33 (52)	42 (63)	<.001 †
Primary treatment						
Radical prostatectomy	216 (44)	129 (42)	33 (32)	14 (22)	14 (21)	<.001 †
Radiotherapy	188 (39)	154 (59)	66 (63)	48 (75)	52 (78)	<.001 †
Endocrine treatment	2 (0.41)	3 (0.97)	1 (0.96)	1 (1.6)		
Watchful waiting	80 (17)	23 (7.4)	3 (2.8)	1 (1.6)	1 (1.5)	<.001 †
Radiotherapy & endocrine treatment			1 (0.96)			
Unknown		1 (0.27)				
Prostate-cancer-specific deaths	8 (1.6)	14 (4.5)	17 (16)	14 (22)	19 (28)	

‡ Kruskal Wallis test. † Pearson's Chi-square (x^2) test.

3+4=7 (log rank P=.001). The survival probabilities of CR/IDC- patients with 4+3=7 or higher were significantly lower than of those with Gleason score 6 (log rank P<.001, P=.03 and P<.001 respectively). CR/IDC- Gleason score 4+3=7 patients also had worse survival probabilities than those with CR/IDC- 3+4=7 (P=.03). Although patients with CR/IDC- Gleason score 9-10 had poorer survival probabilities than those with CR/IDC- Gleason score 3+4=7 prostate cancer (log rank P=.001), there was no statistical difference in disease-specific-survival probabilities comparing CR/IDC- Gleason score 9-10 with CR/IDC- Gleason score 4+3=7 and CR/IDC- Gleason score 8 patients (log rank P=.41 and P=.40, respectively). In general, the 15-year disease-specific-survival probabilities were 94% (95% CI 91-97%) in CR/IDC- Gleason score 3+4=7 or higher (n=356) and 67% (95% CI 59-76%) in CR/IDC+ Gleason score 3+4=7 or higher (n=189). Presence of CR/IDC growth affected disease-specific survival regardless of its extent (Figure 1F).

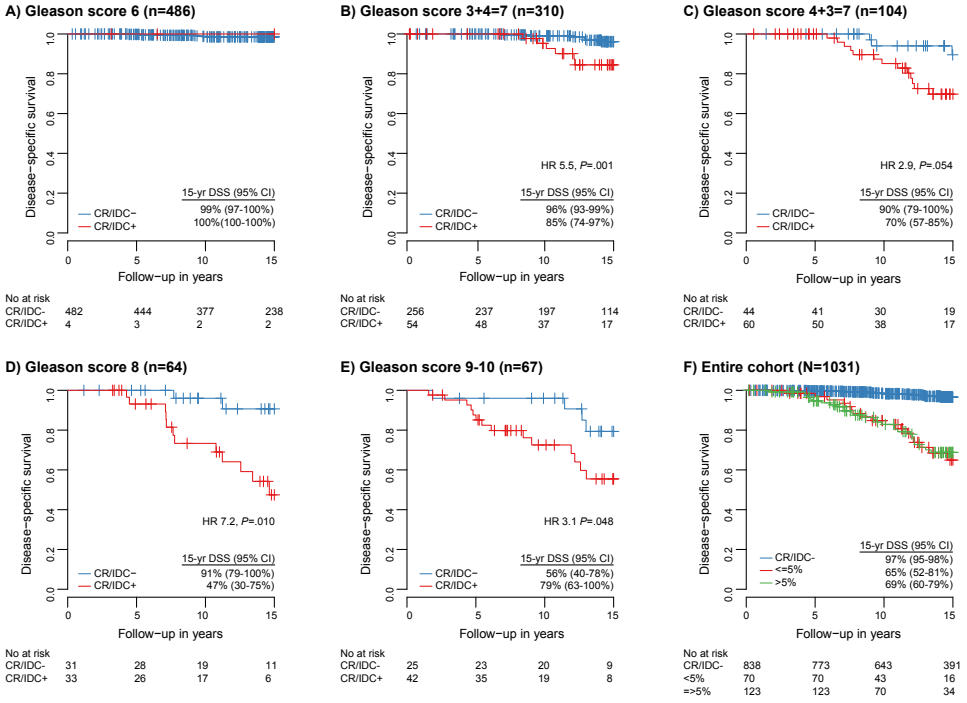

Figure 1. Kaplan-Meier disease-specific survival (DSS) according to Gleason score and CR/IDC status. A) Gleason score 6. B) Gleason score 3+4=7. C) Gleason score 4+3=7. D) Gleason score 8. E) Gleason score 9-10. F) DSS probabilities according to percentage of CR/IDC glands.

In a multivariable model, we analyzed the added prognostic value of CR/IDC status in combination with currently used clinically relevant variables, i.e. age, PSA level, treatment modalities, Gleason score, tumor percentage, and percentage of positive cores. In the model without CR/IDC status the following variables were independently associated with a worse disease-specific survival: PSA level, tumor percentage, percentage of positive cores, and Gleason score 4+3=7 or higher (Table 2). After adding CR/IDC status into the model, Gleason score 4+3=7 and 8 were not independently associated with worse disease-specific survival anymore. We found that the c-index significantly increased from 0.868 to 0.877 after CR/IDC status was added to the model (Likelihood-ratio test P=.001). There was no statistically significant interaction between CR/IDC status and treatment (Likelihood-ratio test, P=.14) or CR/IDC status and Gleason score (Likelihood-ratio test P=.71).

Table 2. Adjusted HRs on time to disease-specific death in a clinical setting: the added value of CR/IDC status (N=1031).

	Model without CR/IDC status			Model with CR/IDC status		
	Adjusted HR	95% CI	P value	Adjusted HR	95% CI	P value
Age (years)	0.99	0.94-1.0	.60	0.99	0.94-1.0	.63
PSA level (log_2)	1.2*	1.0-1.5	.02	1.2*	1.0-1.5	.04
Percentage of positive cores (log_2)	1.8*	1.2-2.6	.006	1.6*	1.0-2.4	.03
Tumor percentage (log_2)	1.5*	1.1-2.1	.02	1.4*	1.0-2.0	.05
Gleason score						
6	Reference			Reference		
3+4=7	1.2	0.48-3.1	.69	0.99	0.38-2.6	.99
4+3=7	3.1	1.2-8.0	.02	1.9	0.67-5.4	.23
8	3.7	1.4-10	.01	2.3	0.78-6.9	.13
9-10	5.1	2.0-13	<.001	3.3	1.2-9.3	.02
CR/IDC+ status				2.6	1.4-4.8	.002
Radical prostatectomy	0.23	0.058-0.92	.04	0.26	0.064-1.0	.05
Radiotherapy	1.3	0.40-4.5	.63	1.4	0.42-4.7	.58

* Per doubling unit

DISCUSSION

The current study showed that CR/IDC status in diagnostic biopsies is associated with a worse disease-specific survival. Adding CR/IDC status to a predictive model resulted in a significantly better discriminative ability. The most interesting finding of our study was the overall good outcome of patients whose biopsies lacked CR/IDC growth, particularly in those with CR/IDC− Gleason score 3+4=7, whose survival did not differ from patients with Gleason score 6 prostate cancer. We additionally found that presence of a limited CR/IDC tumor component (≤5%) in biopsies was already associated with an unfavorable outcome. This finding is in line with the study of Trudel *et al.*, who showed that any amount of large cribriform or intraductal carcinoma was associated with shorter time to biochemical recurrence after radical prostatectomy.[13]

In recent radical prostatectomy studies, intraductal carcinoma and invasive cribriform carcinoma have both been identified as independent prognostic factors.[2-5,11,13] To date, only few studies have analyzed the prognostic value of intraductal carcinoma in pre-treatment diagnostic biopsies.[7-9] They showed that intraductal carcinoma is associated with high-grade and non-organ confined prostate cancer in subsequent radical prostatectomies.[7,8] In addition, Van der Kwast *et al*. demonstrated that intraductal carcinoma was associated with shorter time to biochemical recurrence and distant metastasis after radiotherapy in intermediate- to high-risk prostate cancer patients.[9]

Although CR/IDC status in our predictive model led to significantly better discriminative ability, the absolute c-indices in the models with and without CR/IDC status only differed marginally. CR/IDC status might not affect clinical decision-making in patients with Gleason score 8-10 since these patients will undergo active treatment either way. CR/IDC status could, however, be useful to stratify Gleason score 3+4=7 patients for active surveillance or treatment. A drawback of the current Gleason grading system is its considerable inter-observer variability, in particular when distinguishing Gleason score 3+4=7 from Gleason score 6 prostate cancer.[19,20] Variability in assignment of grade is significantly related to the presence of ill-formed and fused growth patterns; these represented the majority of Gleason score 3+4=7 prostate cancers in this study. Egevad *et al*. found that cribriform growth was not statistically associated with Gleason score inter-observer variability among 337 pathologists.[21] This indicates that CR/IDC status may be a more robust parameter for patient stratification than grading as either Gleason score 6 or 3+4=7.

Although invasive cribriform carcinoma and intraductal carcinoma are two different pathologic entities, they may be related on a pathological and biological level.[22,23] Their morphologic distinction is often difficult requiring immunohistochemical staining for basal

cells. While presence of basal cells is strongly supportive of intraductal carcinoma, lack of basal cells is not pathognomonic for invasive cribriform growth; basal cells can be scattered and not be sampled in the tissue section, which is also known to occur in HGPIN.[24] The use of combined CR/IDC status is practical for pathologic diagnosis since it does not affect prognostic value of separate entities nor requires additional immunohistochemistry. This is also in line with the latest 2014 ISUP recommendations on Gleason grading, in which Epstein *et al*. advised that immunohistochemistry to distinguish invasive cribriform from intraductal carcinoma should only be considered in cases where the results of the studies would change the case's overall grade, for example in cases lacking other Gleason grade 4 patterns.[1]

Several studies have reported on genetic abnormalities related to CR/IDC growth. Qian *et al*. found gain of chromosome 7, 12 and Y, loss of chromosome 8, and extra copies of *c-MYC* in both cribriform HGPIN and invasive cribriform carcinoma, suggesting that these growth patterns are genetically more alike to Gleason grade 5 than Gleason grade 3 or 4 prostate cancer.[25,26] Dawkins *et al*. reported frequent losses of 8p22 and 16q23.1 in intraductal carcinoma.[27] Bettendorf *et al*. found that intraductal carcinoma has more frequent loss of *TP53*, *RB1* and *PTEN*.[28] Using break-points regions to infer phylogenetic relationships, Lindberg *et al*. showed that the clone closely related to the metastases was found in intraductal carcinoma.[29] We hypothesize that both invasive cribriform and intraductal carcinoma are architectural substrates of genetic aberrations associated with aggressive disease behavior. The fact that small CR/IDC components were already associated with worse outcome could be explained by the emergence of aggressive tumor clones irrespective of their volume.

A limitation of the current study is the fact that the original ERSPC biopsy protocol included sextant biopsies, while current biopsy schemes are more extensive and increasingly MRI targeted reducing the chance of sampling error. Future research is needed to confirm that CR/IDC status' prognostic value is similar in contemporary biopsy protocols. Another limitation is the difference in treatment modalities nowadays as compared to the 1990s. Low-risk patients in this study had mostly received active treatment, while active surveillance would have been an acceptable strategy nowadays. The strengths of the current study are its large number of patients with long-term follow-up, the use of disease-specific survival as an outcome measure, and the meticulous pathological review. In conclusion, CR/IDC+ status in prostate cancer biopsies is independently associated with poorer disease-specific survival. Active surveillance criteria may potentially be extended to CR/IDC- Gleason score 3+4=7 prostate cancer, since these patients have similar survival rates as those with Gleason score 6.

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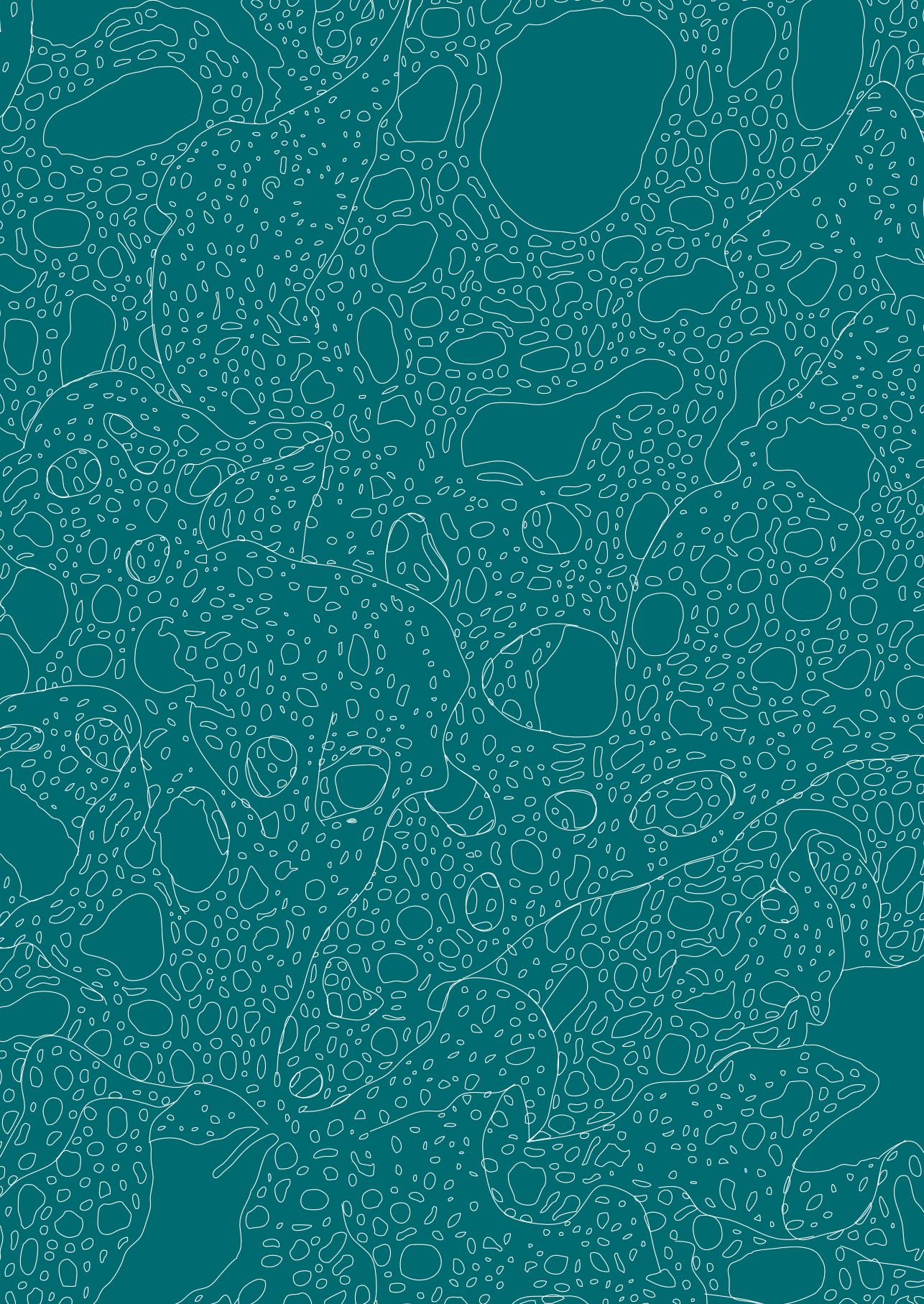

ABSTRACT

Background: Gleason score (GS) 3+4=7 prostate cancer patients with presence of cribriform or intraductal carcinoma (7^+) have a worse disease-specific survival than those without. The aim of this study was to compare the clinico-pathologic characteristics and patient outcomes of men with biopsy GS 3+4=7 without cribriform or intraductal carcinoma (7^-) to those with GS 3+3=6.

Patients and methods: We included all patients from the first screening round of the European Randomized Study of Screening for Prostate Cancer (1993-2000) with a revised GS ≤ 3+4=7 (n=796) following the 2014 ISUP criteria. Relations with biochemical recurrence after radical prostatectomy or radiotherapy were analyzed using log-rank testing and multivariable Cox regression analysis.

Results: In total 486 patients had GS 6 and 310 had GS 7, 54 of whom had GS 7^+ (17%). During a median follow-up of 15 years, biochemical recurrence was seen in 61 (20%) GS 6, 54 (21%) GS 7^- and 22 GS 7^+ patients (41%). Both biopsy GS 7^- and 7^+ patients had significantly higher PSA levels, mean tumor percentage, percentage of positive cores and ≥cT3 than those with GS 6 (all P<.001). GS 7^- patients did not have a poorer biochemical-recurrence-free-survival (BCRFS) after radical prostatectomy than GS 6 patients (log rank P=.13), whereas those with GS 7^+ had (log rank P=.05). In multivariable analyses, biopsy GS 7^- was not associated with poorer BCRFS after radical prostatectomy (HR 1.3, 95% CI 0.67-2.4, P=.47) or radiotherapy (HR 0.88, 95% CI 0.51-1.5, P=.63). GS 7^+ was independently associated with poorer BCRFS after radical prostatectomy (HR 3.0, 95% CI 1.1-7.8, P=.03), but not after radiotherapy (HR 1.2, 95% CI 0.58-2.3, P=.67).

Conclusion: Men with biopsy GS 7^- prostate cancer have similar BCRFS after radical prostatectomy or radiotherapy to those with GS 6 and may be candidates for active surveillance.

INTRODUCTION

The use of prostate-specific antigen (PSA) testing has led to substantially increased detection of insignificant prostate cancers in the past two decades.[1] Active surveillance has gradually emerged as a valuable alternative treatment option for these men. While active surveillance has the benefit of avoiding overtreatment, it also retains the option for active treatment in case of disease progression. Although most active surveillance protocols are limited to Gleason score (GS) 3+3=6, some institutions have extended their criteria to include biopsies containing small amounts of Gleason grade 4.[2-5] Contemporary Gleason grade 4 prostate cancer represents a heterogeneous group of various growth patterns comprising ill-formed, fused, cribriform and glomeruloid glands. While Gleason grade 4 is not sub-classified in daily practice, recent studies have suggested that among Gleason grade 4 growth patterns, cribriform growth is associated with worse clinical outcome, while fused, glomeruloid and ill-formed glands are not.[6-9]

In recent years the clinical relevance of intraductal carcinoma of the prostate – a high-risk lesion defined as malignant epithelium filling large acini or ducts with preservation of basal cells – has been acknowledged. Although not included in the Gleason grading system, intraductal carcinoma has been associated with high GS, advanced tumor stage, biochemical relapse and distant metastasis.[10-15] Intraductal carcinoma can microscopically mimic invasive cribriform carcinoma and immunohistochemistry is often required for their distinction. Recently, our group has shown that biopsy GS 3+4=7 patients without cribriform or intraductal carcinoma (7^-) have comparable disease-specific survival rates to those with GS 3+3=6, while those with cribriform or intraductal carcinoma in their biopsies (7^+) had significantly worse outcomes.[16] Although various studies have shown the adverse prognostic value of invasive cribriform and intraductal growth in GS 7 prostate cancer patients, it is not yet clear to what extent the outcome of men with GS 7^- differs from those with GS 6 prostate cancer. Since GS 3+4=7 prostate cancer patients generally undergo active treatment, identifying low-risk GS 3+4=7 tumors could offer a rationale for active surveillance in this large subgroup of prostate cancer patients. The aim of this study was to compare the clinico-pathologic characteristics and long-term outcomes of men with biopsy GS 3+3=6 or GS 3+4=7 without invasive cribriform or intraductal prostate cancer.

MATERIALS AND METHODS

Patient selection

We identified all 1078 men from the first screening round of the Dutch part of the European Randomized Study of Screening for Prostate Cancer (ERSPC), who had been diagnosed with prostate cancer between November 1993 and March 2000 in Erasmus Medical Center, Rotterdam, The Netherlands. The trial protocol has been published previously.[17,18] After pathologic review of all available slides (n=1055) according to the 2014 International Society of Urological Pathology (ISUP) recommendations, we selected all patients with a highest biopsy GS 3+4=7 or lower for the current study (n=803).[19] Exclusion criteria were presence of a lymph node or distant metastasis at time of diagnosis (n=7). The final selection included 796 patients, 486 of whom had GS 3+3=6 and 310 had 3+4=7.

Pathological evaluation

Three investigators (C.K., I.K., G.v.L.), who were blinded to patient information and outcome, revised all histopathological slides. For each biopsy core we recorded GS, and presence of cribriform pattern or intraductal carcinoma. Since invasive cribriform and intraductal carcinoma often co-exist, and separate classification is challenging, we combined both patterns to one group (CR/IDC). The mean tumor percentage per patient was defined as the sum of total tumor length (mm) divided by the sum of total biopsy length (mm). The label 7^{+} was given to patients with GS 3+4=7 who either had invasive cribriform carcinoma, intraductal carcinoma or both, 7^{-} to those who had neither. Gleason grading was done according to the 2014 ISUP recommendations.[20] The 2009 TNM classification was used to assess pT stage.[21] A positive surgical margin at radical prostatectomy was defined as extension of the tumor into the specimen's inked surface.

Clinical evaluation

Up to February 1997, transrectal ultrasound-guided sextant biopsies were taken based on a combination of digital rectal examination, transrectal ultrasonography, and PSA testing (with a cutoff value of 4.0 ng per milliliter); in 1997, this combination was replaced by PSA testing only. After diagnosis and initial treatment, patients were semi-annually monitored by chart review to assess potential progression and secondary treatments. Biochemical recurrence was defined as a PSA level of ≥0.2 ng/mL assessed at two consecutive time points > 3 months apart after radical prostatectomy or any PSA increase >2 ng/mL higher than the PSA nadir value after radiotherapy.[22] Biochemical-recurrence-free survival (BCRFS) was defined as time after radical prostatectomy or radiotherapy to biochemical recurrence

Statistical analysis

Continuous parameters were analyzed by the Mann-Whitney U test, categorical parameters by the Pearson's Chi-square (x^2) test. Non-normally distributed continuous variables underwent log base 2 transformation such that effects related to a doubling in unit. We estimated survival probabilities using the Kaplan-Meier method. Unadjusted comparisons for survival time were made using log-rank tests with censoring of men lost to follow-up. Crude and adjusted hazard ratios (HRs) for survival time were calculated using Cox proportional hazards regression. Median values of continuous variables were used to create adjusted survival curves. All statistical analyses were performed in R version 3.2.2 (R, Vienna, Austria). Two-sided P values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics

The median (IQR) age was 66 (62-70) years and the median (IQR) follow-up 15 (10-17) years (Table 1). Patients with GS 7 had significantly higher mean PSA levels (P<.001) and more often clinical extra-capsular extension than GS 6 patients (P<.001). Quantitative measurements of tumor involvement, such as mean tumor percentage (P<.001) and percentage of positive cores (P<.001), were also higher in GS 7 patients. Although lymph node and distant metastases were relatively rare in the entire cohort, men with GS 7 had more distant metastases during follow-up than those with GS 6 (5.6% versus 1.9%, P=.009). CR/IDC growth was observed in 54 out of 310 (17%) GS 7 patients. GS 7^{+} patients had higher mean PSA levels (P=.018), percentage of positive cores (P=.015) and tumor percentage (P<.001) than those with 7^{-}. The number of distant metastases in patients with GS 7^{+} was 4.3 fold higher than in GS 7^{-} men (15% versus 3.5%, P=.003). Although GS 7^{-} patients had higher PSA levels (P<.001) and more biopsy tumor involvement (P<.001) than GS 6 patients, the number of observed distant metastases was not statistically different between both groups (P=.25). Overall biochemical recurrence was seen in 61 (20%) GS 6, 54 (21%) GS 7^{-} and 22 GS 7^{+} patients (41%).

Primary treatment

In the 1990s, treatment modalities differed from nowadays with relatively few patients having been selected for active surveillance. In the current study, 80 out of 486 (16%) GS 6 patients were followed by active surveillance, while 84% and 93% of men with GS 6 and 7, respectively, had undergone active treatment. The median (IQR) age of the radiotherapy cohort was 69 years (64-72), whereas the radical prostatectomy cohort was younger having a median (IQR) age of 64 years (60-67, P<.001). Seventy-four out of 342 (22%) radiotherapy patients had clinical extra-capsular extension at diagnosis compared to 27 out of 345 (7.8%) radical prostatectomy patients (P<.001). The mean biopsy tumor involvement did not differ statistically between the radiotherapy and radical prostatectomy cohort (P=.85 and P=.22 for percentage of positive cores and mean tumor percentage, respectively). GS 7 patients had more often received radiotherapy (50% versus 39%; P=.003), whereas watchful waiting was more frequently chosen in GS 6 patients (16% versus 7%; P<.001). Because patient characteristics differed between the two treatment modalities, we decided to focus our further analyses on the separate treatment groups.

Table 1. Patient characteristics entire cohort (n=796).

	GS 6 (n=486)	GS 7(n=310)		GS 7^{-} (n=256)	GS 7^{+} (n=54)		
	Mean (median, IQR) or n(%)		P value*	Mean (median, IQR) or n(%)		P value §	P value ∫
PSA level at diagnosis (ng/mL)	5.8 (4.7, 3.5-6.9)	8.8 (5.8, 4.0-9.0)	<.001 ‡	8.2 (5.7, 4.0-8.4)	11 (7.2, 4.6-12)	.018 ‡	<.001 ‡
Biopsy tumor involvement							
Percentage of positive cores (%)	31 (29, 17-43)	44 (43, 29-57)	<.001 ‡	43 (43, 29-57)	52 (50, 33-67)	.015 ‡	<.001 ‡
Mean tumor percentage (%)	24 (17, 9.5-33)	43 (43, 27-57)	<.001 ‡	41 (39, 23-56)	55 (54, 40-66)	<.001 ‡	<.001 ‡
cT stage							
Tumor identified by biopsy (cT1c)	264 (54)	123 (40)	<.001 †	109 (42)	14 (26)	.03 †	.003 †
Organ-confined disease (cT2)	181 (37)	124 (40)	.48 †	104 (41)	20 (37)	.74 †	.41 †
Extra-capsular extension (cT3)	41 (9)	63 (20)	<.001 †	43 (17)	20 (37)	.002 †	<.001 †
Primary treatment							
Radical prostatectomy	216 (44)	129 (42)	.48 †	112 (44)	17 (31)	.13 †	.92 †
Radiotherapy	188 (39)	154 (50)	.003 †	120 (47)	34 (63)	.05 †	.04 †
Watchful waiting	80 (16)	23 (7.4)	<.001 †	20 (7.8)	3 (5.6)		.002 †
Hormonal treatment	2 (0.41)	3 (0.97)		3 (1.1)			
Unknown		1 (0.32)		1 (0.39)			
Lymph node metastasis	2 (0.41)	8 (2.6)		5 (2.0)	3 (5.6)		
Distant metastasis	9 (1.9)	17 (5.6)	.009 †	9 (3.5)	8 (15)	.003 †	.25 †

‡ Mann-Whitney U test. † Pearson's Chi-square (x^2) test. * Statistical comparisons between Gleason score 3+3=6 and 3+4=7. § Statistical comparisons between Gleason score 3+4=7- and 3+4=7+. ∫ Statistical comparisons between Gleason score 3+3=6 and 3+4=7-. GS = Gleason score.

Radical prostatectomy

Patients with GS 7$^-$ prostate cancer had significantly higher mean PSA levels (P=.018) and more tumor involvement (P<.001) in their biopsies than those with GS 6 (Table 2). In the corresponding radical prostatectomy specimen, extra-prostatic extension (pT3/4) was more frequently observed in biopsy GS 7$^-$ than in GS 6 patients (29% versus 12%, P=.001). Similarly, biopsy GS 7$^-$ patients had more often positive surgical margins than those with GS 6 (29% versus 17%, P=.01). We did not find statistically significant differences in clinical and pathologic characteristics of GS 7$^-$ and GS 7$^+$ patients.

Patients with biopsy GS 7 had a poorer BCRFS than those with GS 6 (log rank P=.03, Figure 1A). After 15 years of follow-up, 87% (95% CI 82-92%) of patients with biopsy GS 6 had been biochemical-recurrence free versus 80% (95% CI 73-87%) of those with GS 7. After stratifying the GS 7 population for CR/IDC growth, GS 7$^+$ patients had a significantly worse BCRFS than those with GS 6 (P=.002) and GS 7- (P=.05, Figure 1). In contrast, BCRFS did not differ statistically between patients with GS 6 and 7$^-$ (log rank P=.13, Figure 1B). BCRFS also differed significantly when comparing GS 6 and 7$^-$ together versus 7$^+$ (P=.006). In multivariable analysis, including clinically relevant variables, PSA level and presence of CR/IDC (7$^+$) were independently associated with a poorer BCRFS, while GS 7$^-$ was not (Table 3, Figure 1C).

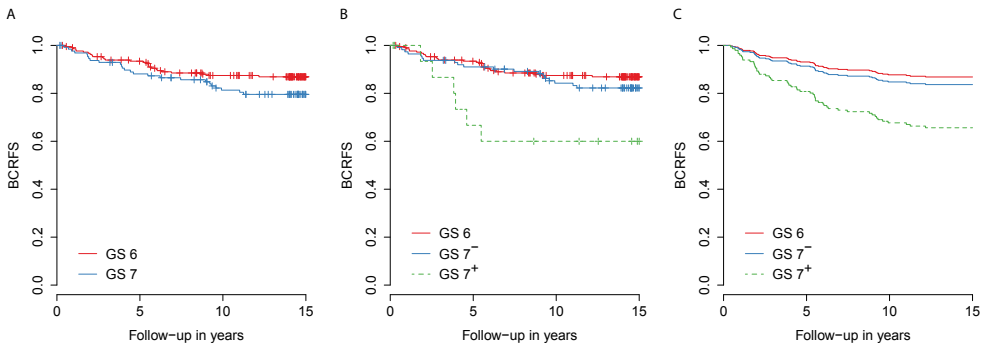

Figure 1. Biochemical-recurrence-free survival after radical prostatectomy of A) Gleason score 6 and 7 patients, B) Gleason score 6, 7$^-$ and 7$^+$ patients. C) Adjusted Biochemical-recurrence-free-survival probabilities of Gleason score Gleason score 6, 7$^-$ and 7$^+$ patients who had undergone radical prostatectomy.

Table 2. Patient characteristics radical prostatectomy cohort (n=345).

	GS 6 (n=216)	GS 7 (n=129)		GS 7^{-} (n=112)	GS 7^{+} (n=17)		
	Mean (median, IQR) or n(%)		P value*	Mean (median, IQR) or n(%)		P value §	P value ∫
PSA level at diagnosis (ng/mL)	5.6 (4.7, 3.5-6.9)	6.8 (5.8, 3.4-8.0)	.005 ‡	6.5 (5.6, 4.0-7.4)	7.4 (6.4, 4.5-8.8)	.23 ‡	.018 ‡
Biopsy tumor involvement							
Percentage of positive cores (%)	33 (33, 17-43)	45 (43, 33-57)	<.001 ‡	44 (43, 33-57)	48 (43, 33-71)	.35 ‡	<.001 ‡
Mean tumor percentage (%)	24 (19, 10-32)	44 (44, 27-60)	<.001 ‡	43 (44, 25-59)	53 (48, 31-70)	.16 ‡	<.001 ‡
cT stage							
Tumor identified by biopsy (cT1c)	107 (50)	57 (44)	.27 †	52 (46)	5 (29)	.20 †	.49 †
Organ-confined disease (cT2)	97 (45)	57 (44)	.79 †	49 (43)	8 (47)	.78 †	.73 †
Extra-capsular extension (cT3)	10 (4.7)	16 (12)	.009 †	12 (11)	4 (24)	.13 †	.04 †
pT stage							
Organ-confined disease (pT2)	187 (87)	91 (71)	<.001 †	80 (71)	11 (65)	.77 †	.001 †
Extra-capsular extension (pT3a)	23 (11)	37 (29)	<.001 †	32 (28)	5 (29)	.91 †	<.001 †
Seminal vesicle invasion (pT3b)	2 (0.93)						
Levator muscle invasion (pT4)	1 (0.46)						
Unknown	3 (1.4)	1 (0.78)			1 (5.6)		
Positive surgical margin	37 (17)	38 (29)	.01 †	33 (29)	5 (29)	.56 †	.01†
Biochemical recurrence	27 (13)	28 (22)		22 (20)	6 (35)		

‡ Mann-Whitney U test. † Pearson's Chi-square (x^2) test. * Statistical comparisons between Gleason score 3+3=6 and 3+4=7. § Statistical comparisons between Gleason score 3+4=7- and 3+4=7+. ∫ Statistical comparisons between Gleason score 3+3=6 and 3+4=7-. GS = Gleason score.

Table 3. Adjusted HRs on time to biochemical recurrence after radical prostatectomy (n=345).

	Radical prostatectomy (n=345)		
	HR	95% CI	P value
Age at diagnosis	1.0	0.96-1.1	.39
PSA level at diagnosis	1.4*	1.0-2.0	.03
Gleason score			
6	Reference		
7^-	1.3	0.67-2.4	.47
7^+	3.0	1.1-7.8	.03
Mean tumor percentage	0.94*	0.74-1.2	.69
Percentage of positive cores	1.3*	0.89-1.9	.17

* Per doubling unit

Radiotherapy

Regardless of CR/IDC growth, biopsy GS 7 patients had higher mean PSA levels, more tumor involvement and clinical extra-capsular extension than those with GS 6 (Table 4). Unlike in the radical prostatectomy cohort, patients with GS 7^+ had significantly higher PSA levels (P=.031), biopsy tumor involvement (P<.001), and more often clinical extra-capsular extension (P=.04) than those with GS 7^-. Although biopsy GS 6 patients had more often cT1 stage compared to GS 7^- patients (50% versus 33%, P=.004), there was no statistical difference in clinical extra-capsular extension (16% versus 24%, P=.10).

Patients with biopsy GS 7 had a poorer BCRFS than those with GS 6 (P<.001, Figure 2A). After 15 years of follow-up, 78% (95% CI 72-85%) of patients with GS 6 had been biochemical-recurrence free versus 62% (95% CI 53-71) of those with GS 7. After stratifying GS 7 patients for CR/IDC growth, 7^+ patients had significantly worse BCRFS than those with GS 6 (log rank P<.001) and 7^- (log rank P=.01, Figure 2B). Biochemical-recurrence free survival probabilities of 7^- patients were significantly worse than those with GS 6 in the crude analysis (log rank P=.04). In multivariable analysis, PSA level, percentage of positive cores and mean tumor percentage were the only variables independently associated with biochemical recurrence, while GS 7^- and GS 7^+ were not (Table 5, Figure 2C).

Table 4. Patient characteristics radiotherapy cohort (n=342).

	GS 6 (n=188)	GS 7 (n=154)		GS 7^{-} (n=120)	GS 7^{+} (n=34)		
	Mean (median, IQR) or n(%)		P value*	Mean (median, IQR) or n(%)		P value §	P value ∫
PSA level at diagnosis (ng/mL)	6.2 (5.0, 3.6-7.6)	10 (6.2, 4.2-11)	.001 ‡	9.0 (5.9, 4.0-9.4)	14 (8.7, 5.1-14)	.031 ‡	.017 ‡
Biopsy tumor involvement							
Percentage of positive cores (%)	29 (32, 17-43)	43 (46, 29-57)	.008 ‡	33 (43, 29-57)	54 (55, 36-64)	<.001 ‡	<.001 ‡
Mean tumor percentage (%)	26 (19, 9.8-36)	43 (44, 27-57)	<.001 ‡	39 (39, 22-54)	56 (55, 41-66)	<.001 ‡	<.001 ‡
cT stage							
Tumor identified by biopsy (cT1c)	95 (50)	47 (30)	<.001 †	40 (33)	7 (21)	.23	.004 †
Organ-confined disease (cT2)	63 (34)	63 (41)	.19 †	51 (43)	12 (35)	.56	.14 †
Extra-capsular extension (cT3)	30 (16)	44 (29)	.007 †	29 (24)	15 (44)	.04	.10 †
Biochemical recurrence	33 (18)	48 (32)		32 (27)	16 (47)		

‡ Mann-Whitney U test. † Pearson's Chi-square (x^2) test. * Statistical comparisons between Gleason score 3+3=6 and 3+4=7. § Statistical comparisons between Gleason score 3+4=7- and 3+4=7+. ∫ Statistical comparisons between Gleason score 3+3=6 and 3+4=7-. GS = Gleason score.

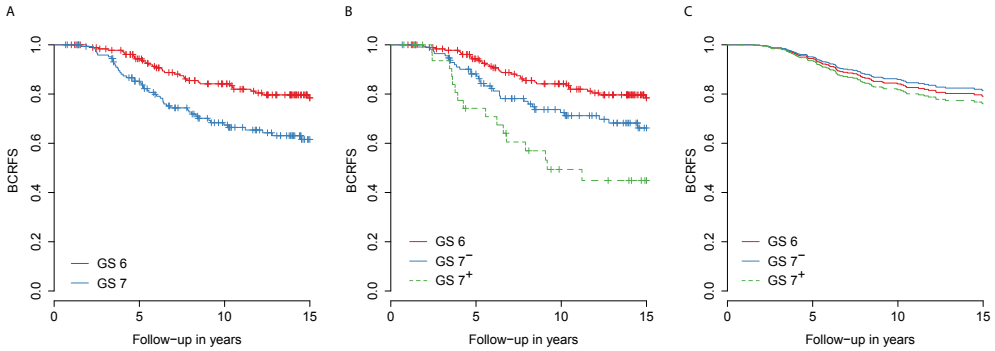

Figure 2. Biochemical-recurrence-free survival after radiotherapy of A) Gleason score 6 and 7 patients, B) Gleason score Gleason score 6, 7^- and 7^+ patients. C) Adjusted Biochemical-recurrence-free-survival probabilities of Gleason score Gleason score 6, 7^- and 7^+ patients who had undergone radiotherapy.

Table 5. Adjusted HRs on time to biochemical recurrence after radiotherapy (n=337).

	HR	95% CI	P value
Age at diagnosis	1.0	0.96-1.1	.71
PSA level at diagnosis	2.2*	1.8-2.8	<.001
Gleason score			
6	Reference		
7^-	.88	0.51-1.5	.63
7^+	1.2	0.58-2.3	.67
Mean tumor percentage	1.4*	1.1-1.8	.006
Percentage of positive cores	1.4*	1.1-1.9	.02

* Per doubling unit

DISCUSSION

Patients with GS 7, regardless of CR/IDC growth, had higher PSA levels, more tumor involvement, and more often clinical extra-capsular extension at biopsy than those with GS 6. We found that GS 7^- patients did not have a statistically different BCRFS and number of distant metastases than those with GS 6, while men with GS 7^+ performed significantly worse. In multivariable analyses, there was no statistically significant difference in BCRFS between GS 7^- and GS 6 prostate cancer patients in both treatment groups, whereas GS 7^+ was associated with a poorer BCRFS after radical prostatectomy. Although previous studies have indicated that both cribriform and intraductal carcinoma are associated with worse outcome in GS 7 patients, our study was specifically focused on the comparison of GS 6 and GS 7^- prostate cancer.[6-11,23] GS 7 prostate cancer patients do not have worse outcome than GS 6, if CR/IDC growth, PSA level and biopsy involvement are taken into account.

An increasing number of studies is providing evidence that invasive cribriform and intraductal carcinoma at prostate biopsy are associated with adverse outcome.[6-11,23] Khani and Epstein reported that most men with biopsy GS 6 with intraductal carcinoma had aggressive tumor features with a 20% rate of disease progression after treatment.[24] Keefe *et al*. reported a 29% (n=30) prevalence of cribriform growth in their cohort of 104 biopsy GS 3+4=7 patients who had all been treated with radical prostatectomy. Of these 30 patients, 18 (60%) had pT3 stage at radical prostatectomy.[25] In our cohort we report both a lower prevalence of CR/IDC growth (13%) in the biopsies and pT3 stage (29%) at radical prostatectomy. The study design may offer an explanation for these differences, as our cohort comprised screen-detected prostate cancers, while Keefe *et al*. included clinically detected prostate cancer patients.[10,25] In our study, CR/IDC growth in GS 7 was independently associated with a poorer BCRFS after radical prostatectomy, but not after radiotherapy.

Van der Kwast *et al*. previously suggested that IDC at biopsy is independently associated with a poorer BCRFS after radiotherapy.[10] This similarly holds true for our study if we only adjust for CR/IDC growth and Gleason score in our multivariable model (data not shown). Our multivariable model, however, also took other variables into account such as PSA and biopsy tumor involvement, after which CR/IDC growth did not have independent prognostic value anymore. In addition, our radiotherapy cohort included patients with cT3 at diagnosis or PSA levels of 20 ng/mL and higher, both of which were exclusion criteria in the study of van der Kwast *et al*.[10]

Gleason grade 4 prostate cancer encompasses a heterogeneous group of growth patterns including ill-formed, fused, glomeruloid and cribriform. The most frequently

reported grade 4 pattern in our entire biopsy cohort (N=1031) was ill-formed (73% in GS 3+4=7 and 74% in GS ≥4+3=7), followed by fused (49% in GS 3+4=7 and 38% in GS ≥4+3=7), cribriform (7.7% in GS 3+4=7 and 20% in GS ≥4+3=7) and glomeruloid (11% in GS 3+4=7 and 13% in GS ≥4+3=7).[16] During the 2005 ISUP conference it was decided that any component of a higher grade in biopsies should be included in the GS.[26] In practice, this means that even a single tumor component interpreted as Gleason grade 4, in an otherwise GS 6 tumor, is increasing the GS to 7. A disadvantage of current Gleason grading is the considerable inter-observer variability, particularly in distinguishing Gleason grade 6 from 3+4=7 prostate cancer.[27-30] In a study among 337 pathologists, Egevad *et al*. found that the percentage of fused and ill-formed glands was inversely correlated with agreement among pathologists, while the cribriform pattern had no significant correlation with inter-observer variability.[30] McKenney *et al*. found that the variability in grading predominantly occurred between tangentially sectioned Gleason grade 3 glands and grade 4 ill-formed glands.[27] In a recent inter-observer study focusing on Gleason grade 4 patterns, our group similarly demonstrated a poor inter-observer reproducibility of fused and ill-formed glands, while agreement was substantial for glomeruloid and cribriform pattern (unpublished data). We therefore propose a place for GS 7$^-$ patients in active surveillance, as their grade may more likely reflect a change in grading practice rather than tumor biology.

Long-term data have shown that active surveillance for low-risk cancers is safe, reporting 10- and 15-year actuarial cause-specific survival rates of 98 and 94%, respectively.[31] This evidence does, however, not yet apply to intermediate-risk patients, such as those with GS 3+4=7. Ploussard *et al*. found in 2323 patients with biopsy GS 3+4=7 that nearly half of the patients had unfavorable disease at radical prostatectomy, defined by pathologic GS ≥4+3 (21%), pT3-4 stage (37%), or both.[32] In GS 3+4=7 patients that met the Prostate cancer Research International: Active Surveillance (PRIAS) criteria for active surveillance (cT ≤T2, PSA ≤10 ng/ml, PSA density <0.2 ng/ml/ml, ≤ 2 positive biopsy cores) the rate of unfavorable disease decreased to 31% (19% GS ≥4+3 and 22% pT3-4 stage).[32] Radical prostatectomies that had been performed in men meeting the PRIAS criteria showed upstaging to Gleason score ≥4+3=7 in 14%-19% and pT3 stage in 10%-19% together with 5-year biochemical-free-survival rates of 91%.[33,34] The comparable rate of upgrading and upstaging in biopsy GS 6 and 7$^-$ patients meeting active surveillance criteria is in line with our findings, and forms a rationale for including low-risk 7$^-$ subpopulations in active surveillance protocols.

A limitation of the current study is that the original ERSPC biopsy protocol included sextant biopsies, while current biopsy schemes are more extensive and increasingly MRI targeted reducing the chance of sampling error. Another limitation is the difference in treatment modalities nowadays as compared to the 1990s. Patients eligible for active

surveillance nowadays all had undergone radical prostatectomy in this study. In addition, the radiotherapy dose given during the 1990s was lower (66-68 Gy) and less efficient as the current radiotherapy protocols (78 Gy).[35] Finally, we were not able to compare our revised biopsy GS according to the ISUP 2014 with the GS at radical prostatectomy, because the prostatectomy GS had been assigned prior to the 2005 ISUP modification and specimens were unavailable for review. The strengths of the current study are its large number of patients with long-term follow-up, the meticulous pathological review of the biopsies, and the focus on GS 3+3=6 and 3+4=7 patients.

In conclusion, men with biopsy GS 7^{-} prostate cancer have similar BCRFS after radical prostatectomy or radiotherapy to those with GS 6 and may be candidates for active surveillance as long as other inclusion criteria such as on PSA and tumor volume are met.

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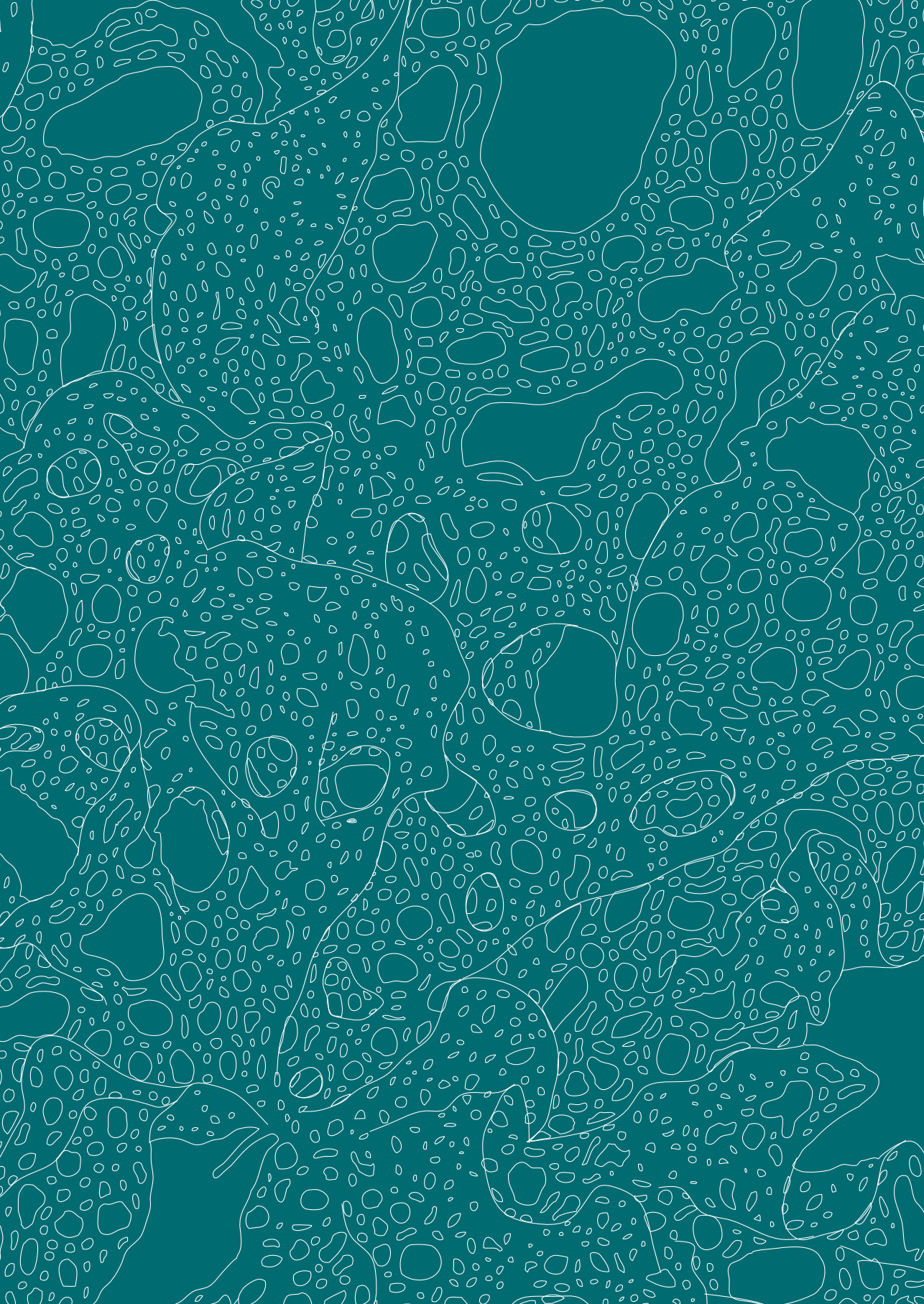

ABSTRACT

Background: Relative increase of grade 4 and presence of invasive cribriform and/or intraductal carcinoma have individually been associated with adverse outcome of Gleason score 7 prostate cancer. The objective of this study was to investigate the relation of percentage Gleason grade 4 (%GG4) and invasive cribriform and/or intraductal carcinoma in Gleason score 3+4=7 prostate cancer biopsies.

Patients and methods: We reviewed 1031 prostate cancer biopsies from the European Randomized Study of Screening for Prostate Cancer. In total 370 men had Gleason score 3+4=7. The relation of invasive cribriform and/or intraductal carcinoma and %GG4 with biochemical recurrence-free survival (BCRFS) after radical prostatectomy (n=146) and radiation therapy (n=195) was analysed using Cox regression.

Results: Invasive cribriform and/or intraductal carcinoma occurred in 7/121 (6%) patients with 1-10% GG4, 29/131 (22%) with 10-25%, and 52/118 (44%) with 25-50% GG4 (P<.001). In crude analysis, both invasive cribriform and/or intraductal carcinoma (HR 2.72; 95% CI 1.33-5.95; P=.006) and 10-50% GG4 (HR 2.43; 95% CI 1.10-5.37; P=.03) were associated with BCRFS after prostatectomy. In adjusted analysis, invasive cribriform and/or intraductal carcinoma was an independent predictor for BCRFS (HR 2.40; 95% CI 1.03-5.60; P=.04) after prostatectomy, while percentage %GG4 (HR 1.00; 95% CI 0.97-1.03; P=.80) was not. While invasive cribriform and/or intraductal carcinoma (HR 2.58; 95% CI 1.59-4.21; P<.001) performed better than 10-50% GG4 (HR 1.24; 95% CI 0.67-2.29; P=.49) for prediction of BCRFS after radiation therapy, both parameters were insignificant in analysis adjusted for PSA (P=.001), positive biopsies (P<.001) and tumor volume (P=.05).

Conclusion: Increased %GG4 is associated with invasive cribriform and/or intraductal carcinoma in GS 3+4=7 prostate cancer biopsies. Invasive cribriform and/or intraductal carcinoma is an independent parameter for BCR after prostatectomy, while %GG4 is not. Presence of invasive cribriform and/or intraductal carcinoma has to be included in pathology reports and should act as exclusion criterion for active surveillance.

INTRODUCTION

The Gleason score (GS) is an important pathologic parameter for risk stratification and therapeutic decision-making in prostate cancer patients. While many patients with GS 6 are eligible for surveillance, active treatment is generally preferred in men with GS 3+4=7 cancer. GS 3+4=7 prostate cancer shows considerable heterogeneity in pathologic features, molecular background and clinical outcome. For optimal individual therapeutic decision-making, therefore, risk stratification of men with GS 3+4=7 prostate cancer is crucial.

Since GS at biopsies is determined by adding the predominant and highest Gleason grade, GS 3+4=7 encompasses cancer with variable quantities of Gleason grade 4 patterns ranging from less than 5% to up to 50%.[1,2] The risk of biochemical recurrence (BCR) after radical prostatectomy is incremental with the percentage of Gleason pattern 4 at the surgical specimen.[3-5] On the other hand, it has been shown that cribriform pattern Gleason grade 4 is associated with BCR and disease-specific death.[5-9] Finally, presence of intraductal carcinoma of the prostate (IDC-P), representing a malignant epithelial proliferation within pre-existent dilated glandular structures, is a marker for aggressive disease behavior.[7,9-11] Invasive cribriform Gleason grade 4 prostate cancer and intraductal carcinoma show overlapping morphologic features, and might be difficult to differentiate without basal cell immunohistochemistry. For practical purposes, therefore, we have labeled invasive cribriform and intraductal growth pattern as one group.[7]

While Gleason grade 4 tumor percentage (%GG4) and presence of invasive cribriform and/or intraductal carcinoma are both of clinical importance for risk stratification of GS 3+4=7 patients, it is unclear how both pathologic parameters are related and whether they both provide independent clinical information. The aim of the current study was to analyze the relation between both parameters on diagnostic biopsies, and to determine their relevance in predicting clinical outcome in GS 3+4=7 prostate cancer patients.

METHODS

Patient selection

We included all 1078 men from the first screening round of the Dutch part of the European Randomized Study of Screening for Prostate Cancer (ERSPC), who had been diagnosed with prostate cancer between November 1993 and March 2000 in Erasmus Medical Center, Rotterdam, The Netherlands. The trial protocol has been published previously.[12,13] After pathologic review of all available slides (n=1054) and exclusion of men with metastatic disease at time of diagnosis (n=23), we selected patients with overall biopsy GS 3+4=7 for the current study (n=370).

Pathological evaluation

Three investigators (C.K., I.K., G.v.L.), who were blinded to patient information and outcome, revised all pathological slides in common sessions. For each biopsy core we recorded GS according to the 2014 ISUP recommendations, total biopsy length, total tumor length, estimated Gleason grade 4 tumor percentage, presence of invasive cribriform pattern and intraductal carcinoma.[2] Since distinction of invasive cribriform and intraductal carcinoma lacks clinical relevance in the majority of cases and might be difficult, if not impossible without the use of immunohistochemistry, we labeled both patterns as one group (CR/IDC) as was also suggested by the ISUP 2014 consensus conference (Figure 1).[2,7] Mean tumor percentage per patient was defined as the sum of total tumor length (mm) divided by the sum of total biopsy length (mm). Percentage Gleason grade 4 (%GG4) was determined by dividing the total length of invasive Gleason grade 4 and intraductal carcinoma by the total tumor length in all biopsies.

Clinical follow-up

After diagnosis and initial treatment, patients were semi-annually monitored by chart review to assess potential progression and secondary treatments. BCR was defined as a Prostate Specific Antigen (PSA) levels of ≥0.2 ng/mL assessed at two consecutive time points > 3 months apart after radical prostatectomy, or any PSA increase >2 ng/mL higher than the PSA nadir value after radiation therapy.[14]

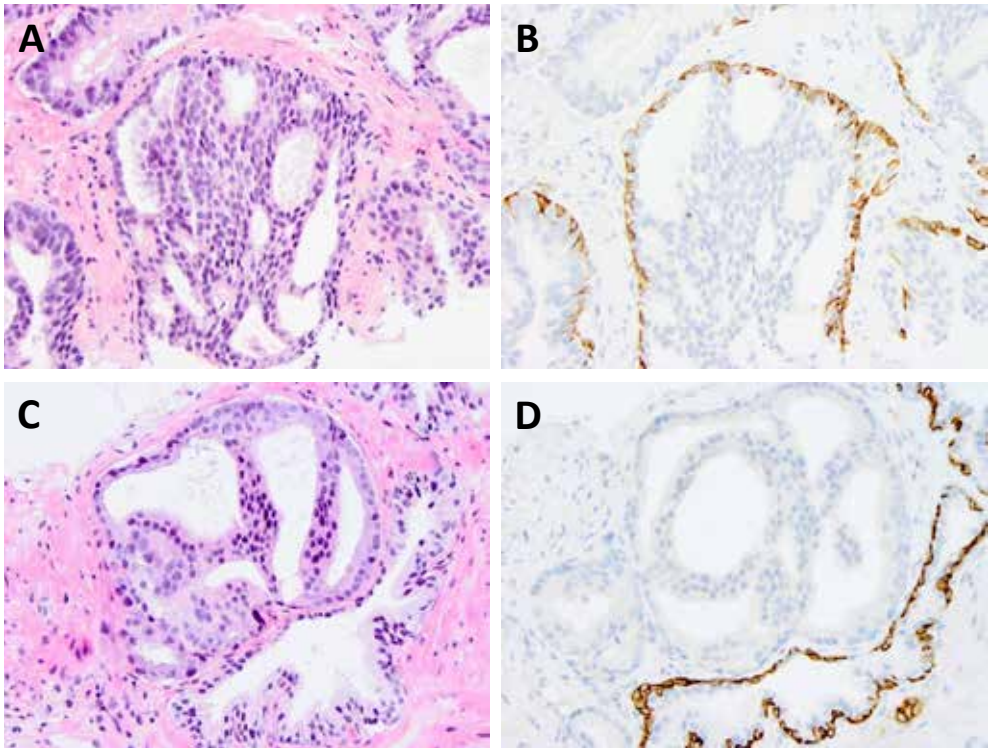

Figure 1. Intraductal carcinoma of the prostate consisting of a dilated gland filled with a malignant cribriform epithelial proliferation with a continuous pre-existent basal cell layer (A, B). Invasive cribriform Gleason grade 4 prostate cancer consists of a malignant cribriform proliferation without basal cell layer (C, D). Hematoxylin & eosin (HE; A, C), high-molecular weight keratin (34BE12; B, D). Original magnifications, 200x.

Statistical analysis

For comparison of %GG4 with clinical and pathologic parameters we grouped the cases as follows: >0% and <10%, ≥10% and <25%, and ≥25% and <50% Gleason grade 4 pattern. Continuous parameters were analyzed by the Kruskal-Wallis test, categorical parameters by the Pearson's Chi-square (x^2) test. We estimated survival probabilities using the Kaplan-Meier method. Unadjusted comparisons for survival time were made using log-rank tests with censoring of men lost to follow-up. Crude and adjusted hazard ratios (HRs) for survival time were calculated using Cox proportional hazards regression. All statistical analyses were performed in SPSS version 21 (IBM, Chicago, Illinois). Two-sided P values of <.05 were considered statistically significant.

RESULTS

Patient characteristics

A total of 370 patients with overall biopsy GS 3+4=7 prostate cancer were identified (Table 1). One hundred and twenty one patients (33%) had less than 10% GG4 component, 131 men (35%) had 10-25% GG4 and 118 (32%) had 25-50% GG4. Age (P=.001), PSA (P<.001) and biopsy tumor volume (P=.001) were all significantly higher in tumors with a greater %GG4. The mean percentage of positive biopsy cores was higher in men with <10% GG4, in whom only four patients had one positive biopsy core (P=.01).

The primary therapeutic interventions for the entire cohort were radical prostatectomy (n=146; 39%), radiation therapy (n=195; 53%), watchful waiting (n=25; 7%) and endocrine therapy (n=3; 1%). Radiation therapy was performed more often (P=.02) and radical prostatectomy less frequently (P=.04) in patients with higher %GG4. Prostate cancer-specific death occurred in 4 (3%), 6 (5%) and 13 (11%) men with <10%, 10-25% and 25-50% GG4 component, respectively (log rank, P=.02).

Invasive cribriform and/or intraductal carcinoma was observed in 88 GS 3+4=7 patients (24%). Invasive cribriform and/or intraductal carcinoma was present in 7/121 (6%) men with less than 10% GG4, in 29/131 (22%) men with 10-25% GG4 and 52/118 (44%) men with 25-50% GG4 pattern (P<.001). Mean PSA level in men with invasive cribriform and/or intraductal carcinoma was 12.9 ng/mL (median 5.2; IQR 8.7-13.7 ng/mL) and 8.5 ng/mL (median 4.0; IQR 5.8-8.7 ng/mL) in men without (P=.001). Biopsy tumor volume was 56% (median 40%; IQR 55-70%) and 41% (median 23%; IQR 39-56%) in men with and without invasive cribriform and/or intraductal carcinoma (P<.001), respectively. Mean %GG4 was 28% (median 14%; IQR 24-33%) in men with invasive cribriform and/or intraductal carcinoma and 16% (median 6%; IQR 12-24%) in those without (P<.001).

Table 1. Clinical and pathologic characteristics of Gleason score 3+4=7 biopsies. Mean (median; IQR) or absolute number (%) are given.

Parameter	Percentage Gleason grade 4			P value
	0-10%	10-25%	25-50%	
Number	121	131	118	
Age	65 (66; 61-70)	67 (68; 63-72)	68 (69; 65-72)	.001
PSA (ng/mL)	7.8 (5.2; 3.7-7.1)	9.2 (5.9; 4.2-9.0)	11.7 (8.5; 5.4-13.4)	<.001
% positive biopsies	51 (50; 33-67)	44 (43; 29-57)	48 (43; 29-67)	.01
% tumor volume	39 (37; 25-52)	44 (45; 27-59)	50 (51; 34-65)	.001
CR/IDC	7 (6%)	29 (22%)	52 (44%)	<.001
Therapy				
Radical prostatectomy	58 (48%)	50 (38%)	38 (32%)	.04
Radiation therapy	52 (43%)	71 (54%)	72 (61%)	.02
Endocrine therapy	1 (1%)	2 (2%)	0	.41
Watchful waiting	9 (7%)	8 (6%)	8 (7%)	.92
Unknown	1 (1%)	0	0	
Disease-specific death	4 (3%)	6 (5%)	13 (11%)	.02

PSA: Prostate Specific Antigen; CR/IDC: invasive cribriform and/or intraductal carcinoma

Radical prostatectomy

The mean follow-up after radical prostatectomy was 14.6 years (median 15.5; IQR 14.0-17.2 years), with 35/146 (24%) men experiencing BCR after 5.8 years (median 4.4; IQR 2.0-9.2 years). BCR occurred more often in men with 10-25% (log rank, P=.04) and 25-50% GG4 (log rank, P=.03) than in those with <10% GG4, but was not statistically different between the three groups (log rank for trend, P=.08). Presence of invasive cribriform and/or intraductal carcinoma was associated with post-operative BCR (log rank, P=.004). In bivariate Cox regression analysis, invasive cribriform and/or intraductal carcinoma (HR 2.73; 95% CI 1.22-6.10; P=.04) was associated with biochemical recurrence-free survival (BCRFS), while %GG4 as continuous parameter (HR 1.00; 95% CI 0.98-1.03; P=.99) was not. Adjusted analysis for age, PSA, percentage positive biopsies, tumor volume revealed invasive cribriform and/or intraductal carcinoma (P=.04) as the only independent parameter for BCRFS after radical prostatectomy (Table 2).

Table 2. Crude and adjusted Cox regression analysis for time to biochemical recurrence after radical prostatectomy.

	Univariate HR (95% CI)	P value	Multivariable HR (95% CI)	P value
Age	1.03 (0.95-1.11)	.49	1.03 (0.95-1.11)	.54
PSA	1.03 (0.98-1.09)	.31	1.01 (0.95-1.08)	.69
Percentage positive biopsies	3.68 (0.85-15.95)	.08	2.27 (0.51-10.09)	.28
Tumor volume	2.20 (0.50-9.76)	.30	1.71 (0.34-8.50)	.51
Percentage GG4	1.01 (0.99-1.04)	.29	1.00 (0.97-1.03)	.80
CR/IDC	2.72 (1.33-5.95)	.006	2.40 (1.03-5.60)	.04

PSA: Prostate Specific Antigen; CR/IDC: invasive cribriform and/or intraductal carcinoma

Since recent surveillance protocols include GS 3+4=7 patients with low amounts of GG4 pattern, we also analyzed the predictive value of dichotomized %GG4.[15,16] In crude regression analysis, men with 10-50% GG4 were at elevated risk for BCRFS as compared to those <10% GG4 (HR 2.43; 95% CI 1.10-5.37; P=.03). In bivariate analysis, presence of invasive cribriform and/or intraductal carcinoma (HR 2.33; 95% CI 1.12-4.84; P=.02) was predictive for BCRFS, while 10-50% GG4 did not meet conventional measures of statistical significance (HR 2.12; 95% CI 0.95-4.74; P=.07).

Radiation therapy

The mean follow-up after radiation therapy was 11.9 years (median 13.1; IQR 8.4-15.9 years). In total, 72 out of 195 (37%) men experienced BCR after 5.7 years (median 4.9; IQR 3.4-7.6 years). BCR occurred more frequently in patients with higher %GG4 (log rank P=.02) and in men with invasive cribriform and/or intraductal carcinoma (log rank, P<.001). In bivariate Cox regression analysis, invasive cribriform and/or intraductal carcinoma (HR 2.43; 95% CI 1.49-4.00; P<.001), but not %GG4 (HR 1.01; 95% 1.00-1.03; P=.14) was associated with BCRFS. Adjusted analysis showed that PSA (P=.001), number of positive biopsies (P<.001) and tumor volume (P=.05) were independently predictive BCRFS after radiation therapy, while %GG4 (P=.19) and invasive cribriform and/or intraductal carcinoma (P=.53) were not (Table 3). Dichotomization of %GG4 revealed no statistically significant difference in BCRFS between men with <10% and 10-50% GG4 (HR 1.67; 95% CI 0.93-3.00; P=.09). Bivariate analysis showed that invasive cribriform and/or intraductal carcinoma (HR 2.58; 95% CI 1.59-4.21; P<.001) was predictive for BCRFS, while 10-50% GG4 was not (HR 1.24; 95% CI 0.67-2.29; P=.49).

Table 3. Crude and adjusted Cox regression analysis for time to biochemical recurrence after radiation therapy.

	Univariate HR (95% CI)	P value	Multivariable HR (95% CI)	P value
Age	1.00 (0.95-1.04)	.90	1.00 (0.95-1.06)	.89
PSA	1.05 (1.04-1.07)	<.001	1.03 (1.01-1.04)	.001
Number positive biopsies	20.10 (7.70-52.51)	<.001	8.55 (2.78-26.32)	<.001
Tumor volume	7.55 (2.69-21.23)	<.001	3.24 (1.00-10.53)	.05
Percentage GG4	1.02 (1.01-1.04)	.009	1.01 (0.99-1.04)	.19
CR/IDC	2.73 (1.72-4.35)	<.001	1.20 (0.68-2.13)	.53

PSA: Prostate Specific Antigen; CR/IDC: invasive cribriform and/or intraductal carcinoma

DISCUSSION

Recent studies have indicated that relative quantity of GG4 pattern and presence of invasive cribriform and/or intraductal carcinoma are promising parameters for risk stratification of GS 3+4=7 prostate cancer patients. In this study, we demonstrated that increased GG4 pattern was strongly associated with presence of invasive cribriform and/or intraductal carcinoma. Patients with <10% GG4 had invasive cribriform and/or intraductal carcinoma in 6%, while it was present in 44% of men with 25-50% GG4. In bivariate analysis, invasive cribriform and/or intraductal carcinoma was an independent parameter for BCR after radical prostatectomy and radiation therapy, while %GG4 as nominal or dichotomized parameter was not. In adjusted analysis, invasive cribriform and/or intraductal carcinoma was an independent parameter for BCR after radical prostatectomy, but not after radiation therapy. These results indicate that the worse outcome of men with high %GG4 might be explained by more frequent presence of invasive cribriform and/or intraductal carcinoma in this group.

Our results on the clinical relevance of %GG4 and invasive cribriform and/or intraductal carcinoma are in line with previous studies. In a large number of radical prostatectomies, Sauter *et al*. found that increased %GG4 went together with BCR.[4] While %GG4 pattern in GS 3+4=7 biopsies has been associated with adverse features at radical prostatectomy, it was not an independent predictive factor for post-operative BCR.[17,18] Biopsy invasive cribriform growth and/or intraductal carcinoma are related to non-organ confined disease as well as BCR after radical prostatectomy and radiation therapy.[11,19-21] Choy *et al*. found independent prognostic value of both %GG4 and invasive cribriform architecture on radical prostatectomy for BCR.[5] The discordance with the current study might be explained by the fact that we determined %GG4 at diagnostic biopsies instead of radical prostatectomy specimens, and that Choy *et al*. did not include intraductal carcinoma in their analysis.

The clinical relevance of intraductal carcinoma, invasive cribriform carcinoma and percentage Gleason grade 4 is increasingly being acknowledged. The World Health Organization (WHO) and International Society of Urological Pathology (ISUP) recommend that presence of intraductal carcinoma is routinely mentioned in pathology reports and that %GG4 is reported in GS 7 prostate cancer patients.[2,22] Percentage GG4 is currently applied as a novel parameter for inclusion of GS 3+4=7 prostate cancer patients in some active surveillance protocols.[15,16] Yamamoto *et al*. for instance found that GS 3+4=7 patients with less than 5% GG4 on surveillance did not experience metastasis.[23] Although presence of invasive cribriform growth and/or intraductal carcinoma has not been formally acknowledged yet as an exclusion criterion for surveillance protocols, it has been suggested to exclude patients with intraductal

carcinoma from surveillance.[24] The results of our study implicate that invasive cribriform growth and/or intraductal carcinoma might be a more reliable factor for therapeutic stratification than %GG4 only, and should be included in pathology reports.

Considerable inter-observer variability exists in differentiating GS 3+4=7 and GS 6 on biopsies.[25-28] We expect that this variability is mainly present in cases with low %GG4, which has been one of the rationales for including GS 3+4=7 patients with low %GG4 in surveillance protocols.[15,16] Grading variability is most prominent in distinguishing ill-formed and fused GS 7 glands from tangentially sectioned GS 6 glands, while concordance on cribriform growth is generally much better.[25,27] The reproducibility of cribriform growth further supports the potential relevance of invasive cribriform and/or intraductal carcinoma for therapeutic decision-making.

In this study, we applied overall instead of highest GS 3+4=7 as inclusion criterion.[7] Since GS might differ between separate cores of the same biopsy session, the overall GS presumes that all biopsies are part of the same tumor. This means that a patient could have positive biopsies with GS 6, 3+4=7, 4+3=7 or 4+4=8 as long as the total %GG4 is less than 50%. In contrast, highest GS 3+4=7 excludes patients with GS 4+3=7 and GS 4+4=8 in single biopsy cores. We have not selected for highest GS 3+4=7 in this study since this would have excluded patients with overall less than 50% GG4. Other groups have shown improved performance of overall versus highest GS in biopsies, challenging clinical practice to classify patients according to the highest GS.[4,17]

In this study, regression analysis of %GG4 dichotomized at a cut-off 10% performed better than %GG4 as continuous parameter. Post-operative BCR between patients with 25-50% GG4 was not statistically significant from men 10-25% GG4. Such a trend was also found by others, and is probably due to small sample size.[18] Despite the lack of independent prognostic value of %GG4, GG4 quantity might still be of interest for further studies. Since this study with long term follow-up was performed on sextant biopsies in 1990s, it is important to further elaborate on the predictive value of biopsy %GG4 and invasive cribriform and/or intraductal carcinoma in the current era of multiple and MRI-targeted biopsies.

In conclusion, we demonstrate that increased %GG4 is associated with a higher frequency of invasive cribriform and/or intraductal carcinoma in GS 3+4=7 prostate cancer biopsies. Invasive cribriform and/or intraductal carcinoma is an independent parameter for BCR after radical prostatectomy, while %GG4 is not. Therefore, stratification for invasive cribriform and/or intraductal carcinoma could be more reliable for inclusion of GS 3+4=7 prostate cancer patients in active surveillance than %GG4 alone. The presence of invasive cribriform and intraductal carcinoma should therefore routinely be included in pathology reports.

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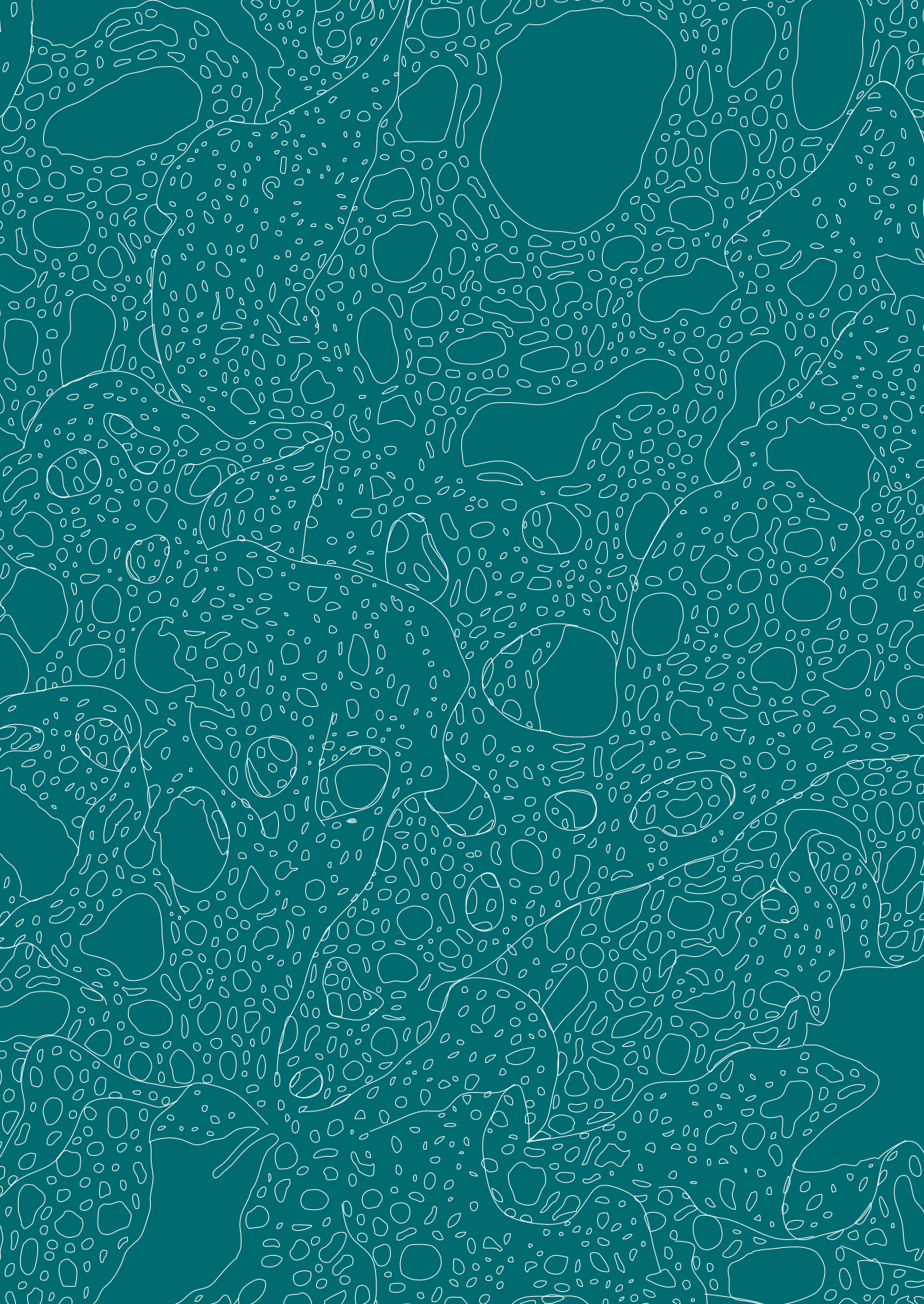

ABSTRACT

Background: To assess the interobserver reproducibility of individual Gleason grade 4 growth patterns.

Patients and methods: Twenty-three genitourinary pathologists participated in the evaluation of 60 selected high-magnification photographs. The selection included 10 cases of Gleason grade 3, 40 of Gleason grade 4 (10 per growth pattern), and 10 of Gleason grade 5. Participants were asked to select a single predominant Gleason grade per case (3, 4, or 5), and to indicate the predominant Gleason grade 4 growth pattern, if present. 'Consensus' was defined as at least 80% agreement, and 'favoured' as 60–80% agreement.

Results: Consensus on Gleason grading was reached in 47 of 60 (78%) cases, 35 of which were assigned to grade 4. In the 13 non-consensus cases, ill-formed (6/13, 46%) and fused (7/13, 54%) patterns were involved in the disagreement. Among the 20 cases where at least one pathologist assigned the ill-formed growth pattern, none (0%, 0/20) reached consensus. Consensus for fused, cribriform and glomeruloid glands was reached in 2%, 23% and 38% of cases, respectively. In nine of 35 (26%) consensus Gleason grade 4 cases, participants disagreed on the growth pattern. Six of these were characterized by large epithelial proliferations with delicate intervening fibrovascular cores, which were alternatively given the designation fused or cribriform growth pattern ('complex fused').

Conclusion: Consensus on Gleason grade 4 growth pattern was predominantly reached on cribriform and glomeruloid patterns, but rarely on ill-formed and fused glands. The complex fused glands seem to constitute a borderline pattern of unknown prognostic significance on which a consensus could not be reached.

INTRODUCTION

The Gleason grading system is one of the most important predictors of prostate cancer progression. In 2005, the International Society of Urological Pathology (ISUP) organized a consensus conference on Gleason grading of prostate cancer.[1] The goal of this meeting was to achieve consensus among leading genitourinary pathologists in specific areas of Gleason grading. At this meeting, large cribriform and ill-formed glands, which are classically described as Gleason grade 3, were redefined as Gleason grade 4.[1] Subsequently, during the 2014 ISUP consensus conference on prostate cancer grading, it was decided that small cribriform and glomeruloid glands should also be considered to be Gleason grade 4.[2] As a result, contemporary Gleason grade 4 prostate cancer represents a heterogeneous group of various growth patterns consisting of ill-formed, fused, cribriform and glomeruloid glands. Although Gleason grade 4 is not subclassified in daily clinical practice, recent studies have suggested that, among Gleason grade 4 growth patterns, cribriform growth is associated with a worse clinical outcome.[3-6]

One of the major limitations of the Gleason grading system is its considerable interobserver variability.[7-19] Fused glands or small glands without lumina may, for instance, be interpreted as tangentially sectioned Gleason grade 3 or as a focal Gleason grade 4 prostate cancer.[15] Despite this interobserver variability, accurate identification of Gleason grade 4 prostate cancer is important for subsequent therapeutic decision-making.[7-19] For instance, patients with Gleason score 3+3=6 prostate cancer are often eligible for active surveillance, whereas patients with 3+4=7 are frequently excluded.[20] To support future studies on Gleason grade 4 architectural subclassification, it is important for individual growth patterns to be well defined and reproducible. We therefore undertook an interobserver variability study among genitourinary pathologists to assess the interobserver agreement regarding individual Gleason grade 4 growth patterns.

MATERIALS AND METHODS

Case selection

Case selection (n = 60) was undertaken by two investigators (C.K. and G.v.L.), and included a representative collection of various Gleason grade 4 growth patterns.[1] The selection was aimed at including 10 Gleason grade 3, 40 Gleason grade 4 (10 per growth pattern) and 10 Gleason grade 5 cases, several of which were deliberately chosen because they showed ambiguous Gleason grade 4 patterns. In this study, we did not include ductal, intraductal or small-cell prostate cancer. Digital images of haematoxylin and eosin-stained slides from selected prostate cancer cases were obtained with a NanoZoomer digital slide scanner (Hamamatsu Photonics, Hamamatsu City, Japan). To ensure that all participants evaluated the same tumour structures, tumour regions of interest were delineated in each case with a yellow line.

Images were incorporated into a digital questionnaire. An international group of 26 genitourinary pathologists were invited to participate in the study. The investigators involved in the case selection (C.K. and G.v.L.) were not included in this group. Participants were asked to select a single predominant Gleason grade per case (3, 4, or 5). If a case was assigned to Gleason grade 4, participants were asked to indicate a single predominant growth pattern (ill-formed, fused, cribriform, or glomeruloid). Participants had the opportunity to add comments by free-text. Finally, we collected basic demographic information from participants. No consensus training preceded the study. The questionnaire, including all cases and instructions, is shown in Appendix S1.

Analysis

Consensus was defined as at least 80% agreement on Gleason grade or Gleason grade 4 subclassification. When 60–80% of the participants agreed, the classification was considered to be 'favoured'. The latter thresholds were chosen arbitrarily. Although participants were asked to select only one predominant grade and/or pattern per case, a combination of grades or patterns, e.g. 'grade 3 and 4' or 'fused and ill-formed', was assigned in a few cases. We considered these combinations as separate categories in the analysis. This also applied to cases that were assigned by free-text to Gleason grade 2, which was not a selection option. In addition, some cases had been assigned to 'Gleason grade 3 cribriform' (n = 4) or 'Gleason grade 3 glomeruloid' (n = 1). The latter cases were considered to be Gleason grade 3 for the analysis. Their growth patterns, however, were included in the Gleason grade 4 pattern analysis.

RESULTS

Participants

Replies were received from 23 genitourinary pathologists (88% response rate) residing in 13 countries: the USA (n = 8), Canada (n = 3), the UK (n = 2), Australia (n = 1), Brazil (n = 1), France (n = 1), Germany (n = 1), Italy (n = 1), Singapore (n = 1), Spain (n = 1), Sweden (n = 1), Switzerland (n = 1), and New Zealand (n = 1). Twenty participants were in academic practice (87%), two were in private practice (8.7%), and one was in academic, community and private practice (4%). The numbers of diagnostic prostate biopsies analysed per year were as follows: 100–250 (n = 1; 4%), 250–500 (n = 7; 30%), 500–1000 (n = 7; 30%), and >1000 (n = 8; 35%).

Gleason grading

Consensus on Gleason grade was reached in 47 (78%) cases: eight were assigned to Gleason grade 3, 35 to Gleason grade 4, and four to Gleason grade 5. In nine cases (15%), a Gleason grade was favoured: four cases were favoured as Gleason grade 3, one as Gleason grade 4, and four as Gleason grade 5. In four cases (7%), there was <60% agreement on Gleason grading (Figure 1). If <80% of participants agreed on Gleason grade, ill-formed (6/13, 46%) and fused (7/13, 54%) were the involved growth patterns. The frequencies of the Gleason grades and grade 4 patterns per case are listed in Table S1.

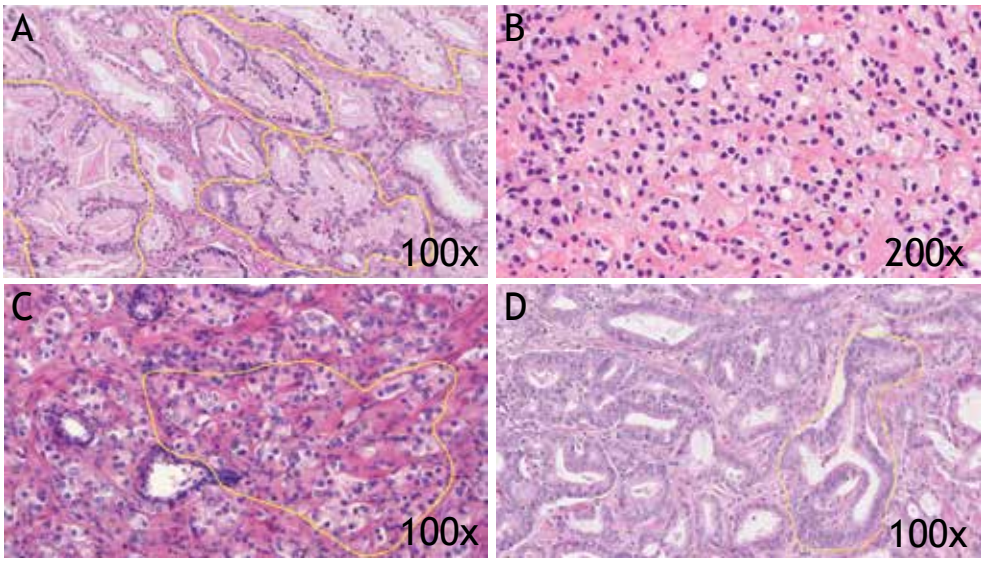

Figure 1. Cases with <60% agreement on Gleason grade. A, Case 22: grade 3, n = 11; grade 4, n = 12 (fused, n = 12). B, Case 24: grade 4, n = 11; grade 5, n = 11; grades 4 and 5, n = 1 (ill-formed, n = 7; fused, n = 2; cribriform, n = 1; fused and ill-formed, n = 2). C, Case 51: grade 4, n = 13; grade 5, n = 8; grades 4 and 5, n = 2 (fused, n = 1; ill-formed, n = 11; fused and ill-formed, n = 2). D, Case 58: grade 3, n = 12; grade 4, n = 11 (fused, n = 7; cribriform, n = 4; cribriform grade 3, n = 1).

Gleason grade 4 patterns

The number of cases in which a Gleason grade 4 pattern was assigned by at least one participant was as follows: fused, n = 41 (68%); cribriform, n = 30 (50%); ill-formed, n = 20 (33%); and glomeruloid, n = 13 (22%). Consensus on a Gleason grade 4 pattern was reached in 13 (37%) of 35 consensus Gleason grade 4 cases: cribriform, n = 7 (54%); glomeruloid, n = 5 (38%); and fused, n = 1 (8%). None of the cases reached consensus for ill-formed glands.

Among the 13 cases in which at least one participant assigned a glomeruloid growth pattern, consensus on a glomeruloid pattern was present in five (38%) cases (cases 12, 18, 25, 29, and 31). The glomeruloid pattern was favoured in two (15%) cases (cases 15 and 38); in both cases, a considerable number of participants preferred cribriform, or a combination of both cribriform and glomeruloid. Consensus on a glomeruloid pattern was found in cases showing relatively small glomerulations, whereas the favoured cases all comprised large glomeruloid formations in which the cribriform pattern was the preferred alternative (Figure 2).

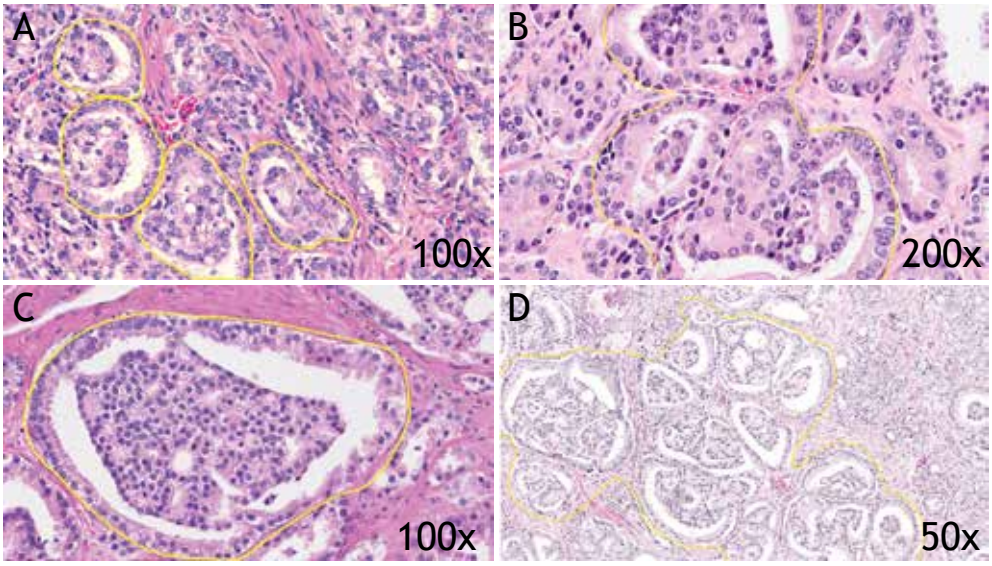

Figure 2. Agreed (A,B) and favoured (C,D) cases with a glomeruloid pattern. A, Case 18: glomeruloid, n = 20; cribriform, n = 1; cribriform and glomeruloid, n = 1. B, Case 29: glomeruloid, n = 19; cribriform, n = 3; fused, n = 1. C, Case 15: glomeruloid, n = 16; cribriform, n = 5; cribriform and glomeruloid, n = 2. D, Case 38: glomeruloid, n = 14; cribriform, n = 4; cribriform and glomeruloid, n = 5.

Among the 30 cases in which at least one participant assigned a cribriform pattern, consensus on a cribriform pattern was reached in seven (23%) cases (cases 4, 9, 17, 27, 30, 43, and 48), two of which (cases 4 and 48) reached 100% agreement. All seven consensus cases had large cribriform glands with multiple punched-out lumina (Figure 3A–D). When a cribriform pattern was favoured (n = 2; 7%), fused glands were also considered by other participants in these cases (cases 16 and 59). Case 16 represented a relatively small gland containing several small mucin-filled spaces and delicate intervening stroma containing capillaries (Figure 3E). Case 59 showed a large glandular proliferation with elongated and round lumina and discrete fibrovascular cores (Figure 3F).

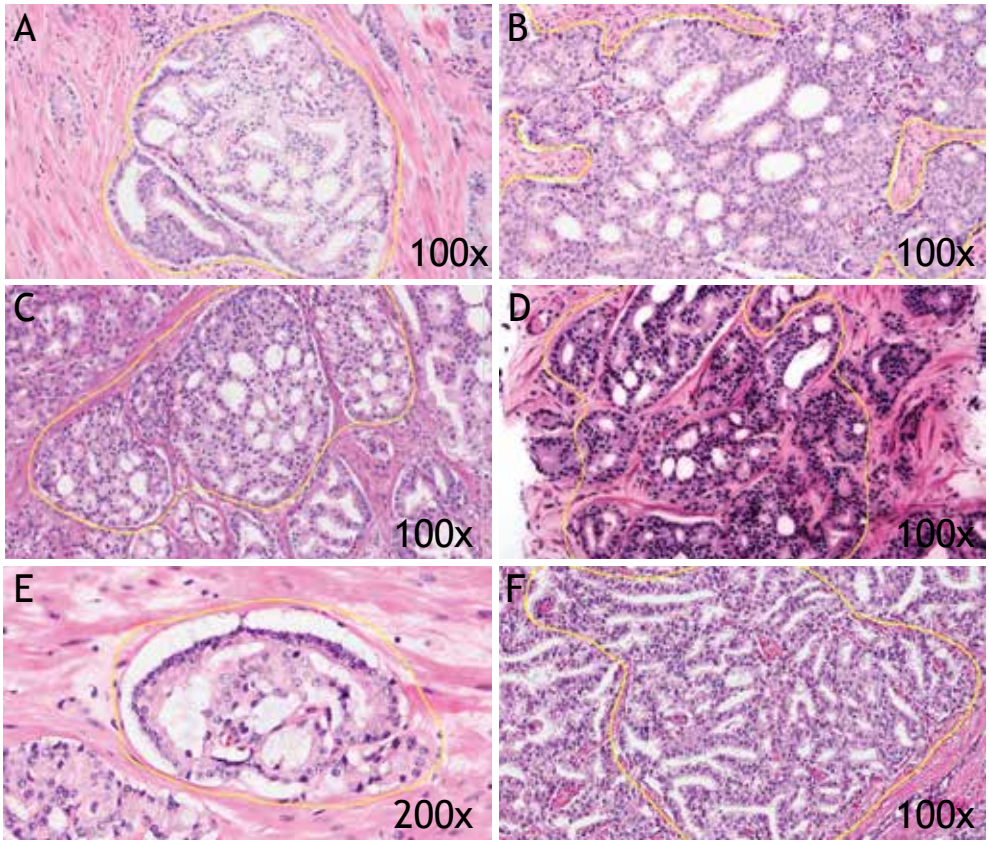

Figure 3. Agreed (A–D) and favoured (E,F) cases with a cribriform pattern. A, Case 4: cribriform, n = 23. B, Case 9: cribriform, n = 21; fused, n = 2. C, Case 27: cribriform, n = 20; glomeruloid, n = 2; cribriform grade 3, n = 1. D, Case 48: cribriform, n = 23. E, Case 16: cribriform, n = 14; fused, n = 6; glomeruloid, n = 1; cribriform and glomeruloid, n = 1; cribriform and fused, n = 1. F, Case 59: cribriform, n = 15; fused, n = 7; cribriform and fused, n = 1.

No consensus was reached on the ill-formed pattern in any of the cases, although this pattern was favoured in five (25%) (cases 2, 5, 7, 39, and 45; Figure 4). In all of these cases, the fused pattern was also considered by other participants. Consensus on the fused pattern was reached in only one (2%) case (case 46), whereas the fused pattern was favoured in four (10%) (cases 19, 32, 54, and 57; Figure 5). In the latter cases, a cribriform pattern was also given by others. Here, the tumour glands had a complex architecture showing anastomosing glands with elongated lumina and delicate intervening stroma and capillaries.

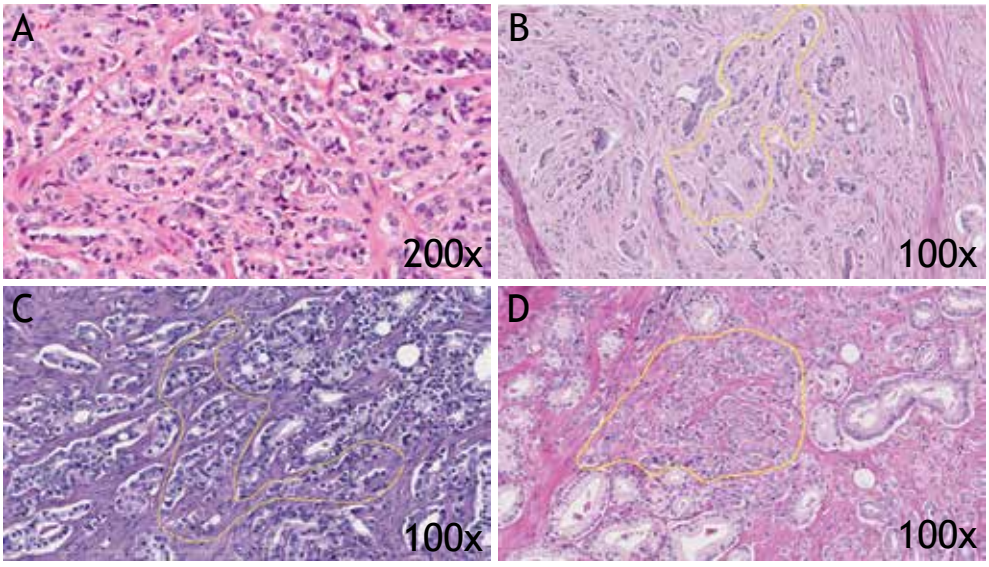

Figure 4. Cases with a favoured ill-formed pattern. A, Case 2: ill-formed, n = 14; fused, n = 7; fused and ill-formed, n = 1. B, Case 5: ill-formed, n = 18; fused, n = 2; fused and ill-formed, n = 1. C, Case 39: ill-formed, n = 16; fused, n = 2; fused and ill-formed, n = 2. D, Case 45: ill-formed, n = 18; fused, n = 3; fused and ill-formed, n = 1.

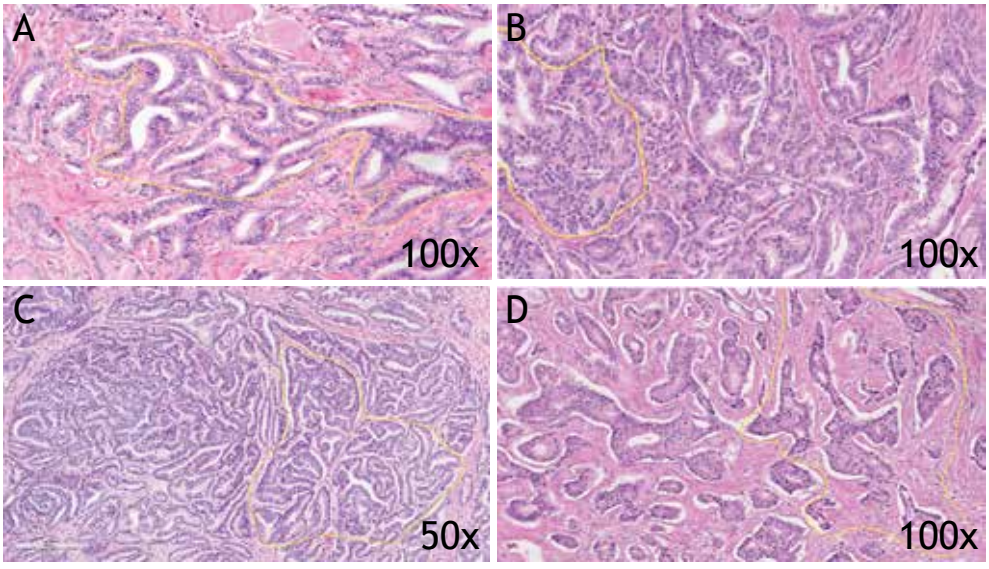

Figure 5. Cases with a favoured fused pattern. A, Case 19: fused, n = 16; cribriform, n = 2; fused and ill-formed, n = 1. B, Case 32: fused, n = 17; cribriform, n = 5; ill-formed, n = 1. C, Case 54: fused, n = 17; cribriform, n = 2; cribriform and fused, n = 1; cribriform and ill-formed, n = 1. D, Case 57: fused, n = 14; cribriform, n = 4; ill-formed, n = 1; fused and ill-formed, n = 1; cribriform and fused, n = 1.

There was <80% agreement on the pattern subclassification in 22 of 35 consensus Gleason grade 4 cases. In these cases, pattern disagreement occurred mostly for fused versus ill-formed and fused versus cribriform. Cases in which no agreement could be reached on a fused or an ill-formed pattern showed glands that were relatively small with an irregular border (cases 3 and 36; Figure 6A,B). The other type of disagreement was characterized by complex epithelial anastomosing glands with delicate intervening fibrovascular cores (‘complex fused), which were alternatively given the designation fused or cribriform pattern (cases 20, 23, 37, 40, 42, and 53; Figure 6C–H). The distribution of the Gleason grade 4 patterns related to disagreement on Gleason grading or grade 4 subclassification are summarized in Figure 7.

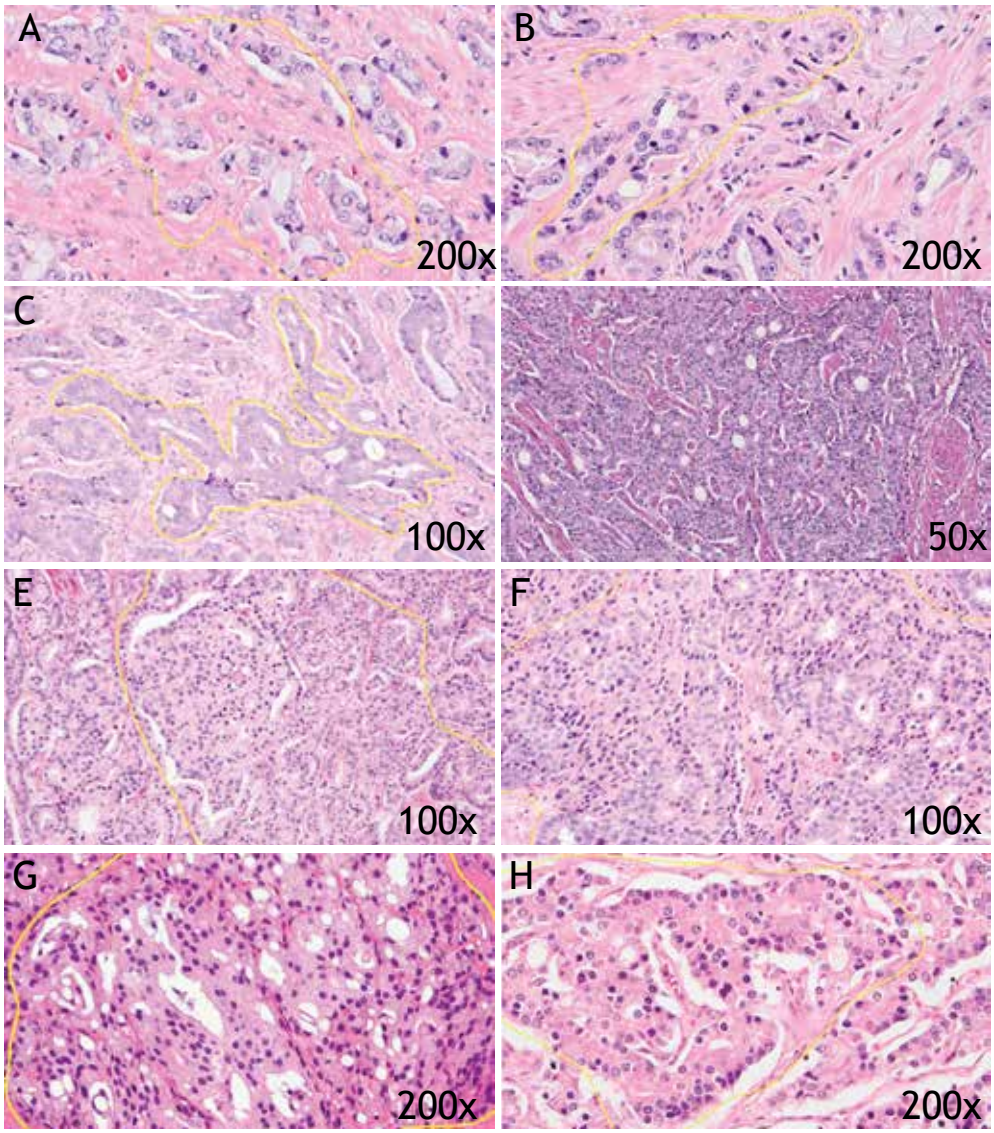

Figure 6. Cases with <80% agreement on Gleason grade 4 subclassification. A, Case 3: ill-formed, n = 13; fused, n = 4; fused and ill-formed, n = 3. B, Case 36: ill-formed, n = 13; fused, n = 4; fused and ill-formed, n = 3. C, Case 23: fused, n = 12; cribriform, n = 7; cribriform and fused, n = 2; fused and ill-formed, n = 1. D, Case 20: fused, n = 13; cribriform, n = 6; cribriform and fused, n = 1; cribriform and fused and ill-formed, n = 1. E, Case 37: cribriform, n = 11; fused, n = 6; glomeruloid, n = 1; fused and glomeruloid, n = 1; cribriform and glomeruloid, n = 1; fused and ill-formed and glomeruloid, n = 1. F, Case 40: cribriform, n = 13; fused, n = 8; fused and ill-formed, n = 1. G, Case 42: cribriform, n = 13; fused, n = 7; cribriform and fused, n = 1. H, Case 53: fused, n = 11; cribriform, n = 10; fused and ill-formed, n = 1; cribriform and ill-formed, n = 1.

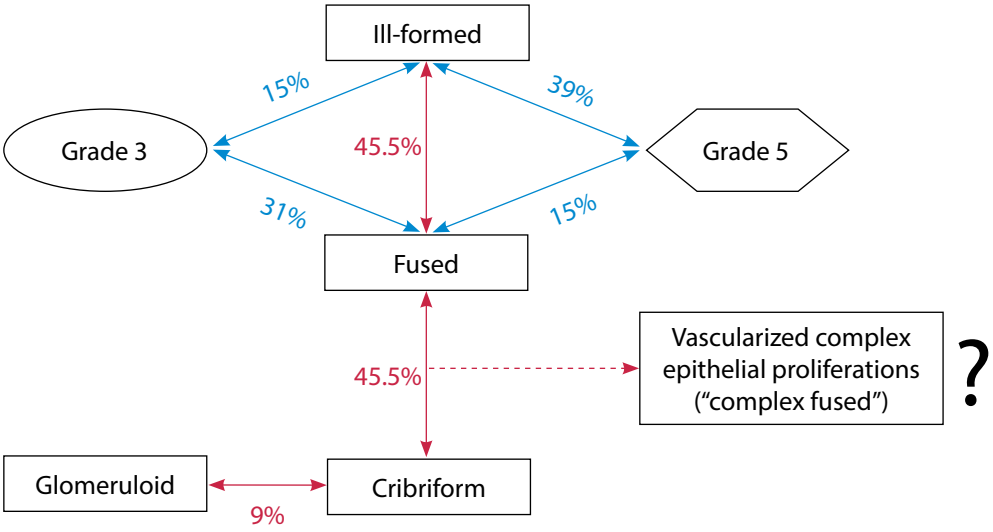

Figure 7. Schematic overview showing types of disagreement in cases with <80% consensus on Gleason grading (n = 13/60, blue arrows) and grade 4 subclassification (n = 22/35, red arrows). The percentages represent the proportion of cases involved in a particular type of disagreement. The ellipse represents Gleason grade 3 pattern, the trapezium Gleason grade 5 pattern, and the rectangles Gleason grade 4 pattern.

DISCUSSION

To facilitate future studies on Gleason grade 4 subclassification, it is important for individual growth patterns to be well defined and reproducible. We therefore initiated an interobserver study among genitourinary pathologists to assess the interobserver agreement regarding individual Gleason grade 4 growth patterns. We found that consensus on a Gleason grade 4 growth pattern was predominantly reached on cribriform and glomeruloid glands, but hardly on ill-formed and fused glands. Although most participants agreed on classifying large epithelial proliferations with multiple punched-out lumina lacking intervening stroma as the cribriform pattern, there was considerable interobserver variation in the subclassification of large complex epithelial proliferations with subtle intervening stroma; these were alternatively regarded as cribriform or fused. To our knowledge, this histological category of 'complex fused' has not been described previously. Also, glomerulations with large intraluminal cribriform proliferations were alternatively regarded as cribriform or glomeruloid.

Fused and ill-formed glands were the two patterns that were most related to Gleason grade variability (3, 4, or 5). Our findings are in line with two previous studies by Egevad *et al.*, who investigated the interobserver reproducibility of Gleason grade 4 patterns among 15 and 337 pathologists, respectively.[21,22] In one of these studies, the percentage of fused and ill-formed glands was inversely correlated with agreement among pathologists, whereas the cribriform pattern had no significant correlation with interobserver variability.[22] Zhou *et al.* recently suggested that adjacent tumour glands play an important role in decision-making in cases showing ambiguous ill-formed patterns.[19] They recommend that >10 poorly formed glands not immediately adjacent to other well-formed glands should be considered to represent ill-formed Gleason pattern 4. In contrast, poorly formed glands that are intermixed with well-formed glands, or ≤5 poorly formed glands, regardless of their location, should be diagnostic features arguing against Gleason pattern 4. Because our study specifically focused on growth pattern subclassification and lacked sufficient adjacent tumour glands, we were unable to make such observations.

Although Gleason grading is subject to interobserver variability, accurate identification of Gleason grade 4 prostate cancer is important for subsequent therapeutic decision-making.[7-19] In many institutes, patients with Gleason score 6 prostate cancer are candidates for active surveillance, whereas patients with Gleason score 7 generally undergo therapeutic intervention.[20] It has been estimated that up to 13% of patients would have been recommended different treatments solely on the basis of pathological re-evaluation of diagnostic biopsies.[13,18] Although the presence of a Gleason 4 pattern is a clinical threshold for active treatment, Gleason grade 4 comprises a heterogeneous tumour group, covering at least

four morphologically distinct patterns. Recent studies have suggested that individual Gleason grade 4 growth patterns are associated with clinical outcome.[3-6] Dong *et al*. found, in a consecutive series of 214 patients, that the presence of cribriform growth was associated with a shorter time to biochemical recurrence and metastasis after radical prostatectomy, whereas fused and ill-formed glands were not.[5] In a cohort of 161 Gleason grade 7 prostate cancer patients, our group also found that the presence of cribriform growth was associated with worse metastasis-free survival and disease-specific survival, whereas fused, glomeruloid and ill-formed glands were not.[6] In a screen-detected cohort of 1031 men, we recently reported similar findings for cribriform growth in diagnostic biopsies.[23] We therefore recommend reporting the presence of cribriform tumour glands in pathology reports, as this pattern appears to confer a less favourable outcome.

The present study has several limitations. Cases were selected by two investigators, who intentionally included both classic and ambiguous cases. For instance, large epithelial proliferations with punched-out lumina and subtle scattered fibrovascular cores, which are difficult to classify as either cribriform or fused, are relatively uncommon. It is therefore difficult to draw conclusions on the general agreement on Gleason grade 4 growth patterns in daily clinical practice. Second, participants were asked to score delineated tissue areas to ensure that categorization discordances were not attributable to the evaluation of different tumour areas. However, the participants were therefore not able to interpret tumour growth patterns in a larger context.

The present study did not make use of a statistical measure of agreement, such as Cohen's κ or Krippendorff's α.[23,24] Because of the study design, in which a Gleason grade 4 subclassification only had to be indicated when Gleason grade 4 was present, we had missing values for grade 4 subclassification when participants assigned a case to Gleason grade 3 or 5. Cohen's κ was therefore not a suitable statistical measure in this context, as it is known to be highly influenced by missing values.[25] Although Cohen's κ is commonly used in the medical literature, its value may not be sufficiently informative, because it relates the proportion of observed agreement to variation in a sample (i.e. relative measure), whereas the clinical question of observer variation in individual cases calls for an absolute measure, i.e. percentage agreement.[26]

In conclusion, consensus on Gleason grade 4 growth pattern was predominantly reached on glomeruloid and cribriform patterns, and rarely on ill-formed and fused patterns. These data indicate that Gleason grade 4 cribriform morphology, which has been associated with a worse clinical outcome, is a reasonably reproducible pattern in Gleason grade 4 prostate cancer. The complex fused glands seem to represent a borderline pattern of unknown prognostic significance on which a consensus could not be reached.

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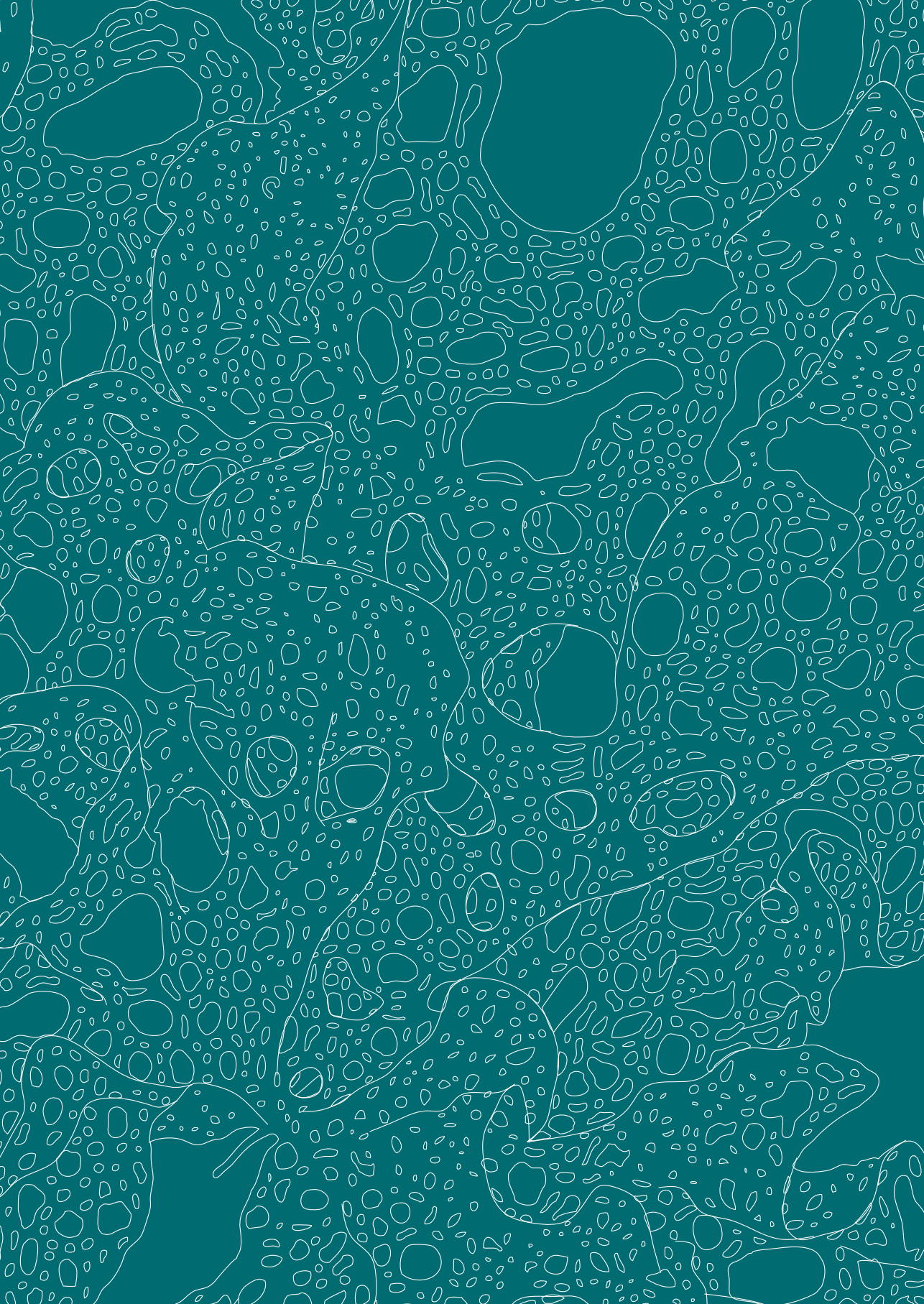


ABSTRACT

Background: Invasive cribriform and intraductal carcinoma (CR/IDC) is associated with adverse outcome of prostate cancer patients. The aim of this study was to determine the molecular aberrations associated with CR/IDC in primary prostate cancer, focusing on genomic instability and somatic copy number alterations (CNA).

Methods: Whole-slide images of The Cancer Genome Atlas Project (TCGA, n=260) and the Canadian Prostate Cancer Genome Network (CPC-GENE, n=199) radical prostatectomy datasets were reviewed for Gleason score (GS) and presence of CR/IDC. Genomic instability was assessed by calculating the percentage of genome altered (PGA). Somatic copy number alterations (CNA) were determined using Fisher-Boschloo tests and logistic regression. Primary analysis were performed on TCGA (n=260) as discovery and CPC-GENE (n=199) as validation set.

Results: CR/IDC growth was present in 80/260 (31%) TCGA and 76/199 (38%) CPC-GENE cases. Patients with CR/IDC and ≥ GS 7 had significantly higher PGA than men without this pattern in both TCGA (2.2 fold; P=0.0003) and CPC-GENE (1.7 fold; P=0.004) cohorts. CR/IDC growth was associated with deletions of 8p, 16q, 10q23, 13q22, 17p13, 21q22, and amplification of 8q24. CNAs comprised a total of 1299 gene deletions and 369 amplifications in the TCGA dataset, of which 474 and 328 events were independently validated, respectively. Several of the affected genes were known to be associated with aggressive prostate cancer such as loss of *PTEN*, *CDH1*, *BCAR1* and gain of *MYC*. Point mutations in *TP53*, *SPOP* and *FOXA1* were also associated with CR/IDC, but occurred less frequently than CNAs.

Conclusion: CR/IDC growth is associated with increased genomic instability clustering to genetic regions involved in aggressive prostate cancer. Therefore, CR/IDC is a pathologic substrate for progressive molecular tumour derangement.

INTRODUCTION

Prostate cancer is heterogeneous with respect to its pathologic features, genetic background and clinical outcome. Clinical-decision making mostly depends upon serum Prostate Specific Antigen (PSA) level, clinical tumor stage, and pathologic biopsy Gleason score (GS) – a grading system based on architectural tumor patterns.[1] While patients with the lowest GS ≤6 (WHO/ISUP group 1) have an excellent patient outcome, those with the highest GS 9-10 (WHO/ISUP group 5) have the worst.[1,2] The clinical outcome of GS 3+4=7 (WHO/ISUP group 2) prostate cancer patients is variable. Improving risk assessment in this subgroup of patients is of clinical relevance as biopsy GS 3+4=7 is an important threshold for active treatment. Recent studies have indicated that, among Gleason grade 4 growth patterns, cribriform growth is mostly associated with a worse clinical outcome.[3-6]

In recent years the clinical relevance of intraductal carcinoma of the prostate (IDC) – a malignant epithelial proliferation filling and extending pre-existent glands – has been acknowledged. Although not included in the Gleason grading system, IDC has been associated with high GS, advanced tumor stage, biochemical relapse and distant metastasis.[7-12] IDC often mimics invasive cribriform carcinoma, and basal cell immunohistochemistry is often necessary for their distinction. Recently, our group has shown that patients with cribriform and/or intraductal carcinoma (CR/IDC), have significantly worse disease-specific survival probabilities than those without, regardless of GS.[13] In addition, patients with focal CR/IDC have similar outcomes as men with extensive CR/IDC, indicating that the mere presence of this growth pattern is already an adverse feature.[13,14]

Although the number of mutational events in prostate cancer is relatively low, copy number alterations (CNAs) are significantly more frequent.[15-24] Several studies have developed molecular prognostic signatures, showing that indolent tumors have relatively few CNAs in contrast to large-scale CNAs in high-grade or metastatic tumours.[16,17,25,26] Both the intra- and inter-tumor heterogeneity, however, pose significant challenges for personalizing treatment in patients with prostate cancer.[27-29] GS 7 prostate cancers, for instance, harbor a wide range of CNA burden varying between <1% and 50%.[26]

Since presence of CR/IDC growth pattern is an independent, adverse clinico-pathologic parameter, we hypothesize that CR/IDC represents a morphological substrate of genomic alterations associated with aggressive disease.[13] The objective of this study was to determine the CNAs and single nucleotide variants (SNVs) associated with CR/IDC using bioinformatics analyses of datasets from The Cancer Genome Atlas Project (TCGA) and the Canadian Prostate Cancer Genome Network (CPC-GENE).

MATERIALS AND METHODS

Pathological review

Via online access (http://cancer.digitalslidearchive.net) and mScope Portal (Aurora Interactive, Montréal, Canada) three investigators with expertise in urogenital pathology (C.K., Th.v.d.K., and G.v.L.) reviewed available whole-slide images of frozen sections of both TCGA (n=260) and CPC-GENE (n=199) cohorts. Both cohorts contained radical prostatectomy specimens without prior hormonal or radiation therapy. Each slide was reviewed for GS, tumor percentage and percentage CR/IDC. Percentage CR/IDC was defined as estimated number of CR/IDC tumor cells divided by the total number of cells present in the tissue slice. Since invasive cribriform and IDC-P were morphologically indistinguishable, they were not scored individually.[13]

Somatic copy number alterations

All statistical analyses were performed in the statistical programming language R v3.2.1 and all genomic coordinates in this manuscript are based on the latest hg19 genome build. Gene-wise $\log_2$ ratios for revised TCGA PRAD samples (based on Affymetrix SNP 6.0 arrays) were retrieved via the TCGA-Assembler R-package.[30] To obtain discrete values, gains or deletions of genetic regions were called if a sample's copy number exceeded the threshold of $\pm\log_2(1.5/2)$. Similarly, a gene-by-sample matrix was obtained for all revised CPC-GENE samples based on Affymetrix OncoScan arrays as described previously.[17] Percent genome altered (PGA) was calculated for both the whole genome (excluding chrX and chrY) and separately for individual chromosome arms as described previously.[17] For chromosome arms, separate PGAs for amplifications and deletions were obtained by dividing the number of bases affected by a deletion/amplification by the number of bases of the respective chromosome arm, taking into account only one DNA strand as PGA does not account for the strand of CNAs. For all values, a Wilcoxon-Mann-Whitney test was performed to test for significant differences between GS categories.

To identify CR/IDC-associated events, the TCGA cohort was used as a discovery set and the CPC-GENE cohort was used for validation. We initially used all CR/IDC positive samples for our analyses, but subsequently limited the CR/IDC group to cases with at least 30% to account for possible signal losses due to dilution effects caused by non-CR/IDC tissue without CNAs. This dilution effect can be envisioned by assuming that CNAs of interest are CR/IDC-associated and corresponding signals therefore mainly originate from the CR/IDC compartment of the tumor. Surrounding non-CR/IDC tissue hence likely does not harbor these CNAs and only contributes to background signal leading to a reduced

signal-to-noise ratio when trying to detect the CNAs in a mixture of both tissues. Prior to analysis, duplicated gene names, known read-throughs, genes on non-random/haplotype chromosomes, as well as genes in pseudoautosomal regions and with missing data were removed. After these filtering steps, 22,350 and 22,420 genes remained for analysis of the TCGA and CPC-GENE cohort, respectively. Next, adjacent genes exhibiting the same CNA profiles were grouped into regions to further reduce the number of tests. Boschloo's exact test (one-sided, R-package 'Exact') was applied to regions with CNAs in at least 10% of all samples to identify events that occurred significantly more often in samples with CR/IDC. Multiple testing correction was performed via false discovery rate (FDR) and regions with a q-value below 0.05 were considered significant. To integrate both cohorts, all genes in regions that were identified as significant in the TCGA cohort were tested in the CPC-GENE cohort. Genes with a q-value below 0.1 were considered validated. A logistic regression was used to assess which individual deletion or amplification events were predictive for CR/IDC status while accounting for PGA and GS as confounding factors. To account for correlations between PGA and individual CNAs, PGA was re-calculated for each event by excluding the chromosome the particular event was located on. Visualization of results was done with BoutrosLab.plotting.general R-package (v5.6.10).

ERG expression, chromothripsis and kataegis

To quantify *ERG* expression in the TCGA cohort, RSEM 'scaled estimates' were obtained via TCGA-Assembler and multiplied by 10^6 to convert them to transcripts per million (TPM). Subsequently a $\log_{10}$ transformation was applied and UCSC transcript uc002yxa.2 was used to estimate *ERG* expression. Deletion events located between *TMPRSS2* and *ERG* were determined by combining deletions of the genes *ETS2*, *BACE2*, *BRWD1*, *PSMG1* and *HMGN1*. For the CPC-GENE cohort, scores for chromothripsis and kataegic regions were computed using the ShatterProof and SeqKat algorithms.[31,32] The maximum values for each sample were used for comparison (Wilcoxon-Mann-Whitney test) to ascertain that despite their rare occurrence, any presence of these phenomena in the CPC-GENE samples could be detected and tested for association with CR/IDC.

Somatic mutations

Automated and curated somatic mutation calls for exome sequencing data from TCGA PRAD samples were obtained via the TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/). Functional events were summarized patient-wise for each gene (*i.e.* multiple mutations in one gene were only counted once per patient, excluding categories 'Silent' and 'RNA'). In addition, non-recurrent events and events that occurred in less than 5% of all tested samples were excluded from further analysis; all remaining gene mutations were tested for significant enrichment in CR/IDC positive samples using Boschloo's exact test (one-sided, R-package 'Exact'). CPC-GENE whole genome sequencing-derived SNVs were filtered to only include functional mutations located in exonic regions and then processed as described above.[32]

RESULTS

Patient characteristics

Patient characteristics of both TCGA (n=260) and CPC-GENE (n=199) cohorts are listed in Table 1. The TCGA cohort included more patients with adverse characteristics than the CPC-GENE cohort, having higher PSA levels (Wilcoxon rank sum test, $P=2.2\cdot10^{-16}$), GS (Pearson's x2 test, $P=4.0\cdot10^{-5}$) and pT stage (Pearson's x2 test, $P=3.1\cdot10^{-9}$), which can be explained by the specific inclusion of clinically intermediate-risk disease in the latter cohort. Moreover, tumor cellularity was higher in TCGA than CPC-GENE (Supplementary Figure 1). Representative prostate cancer samples of GS 6 and GS ≥ 7 are depicted in Figure 1.

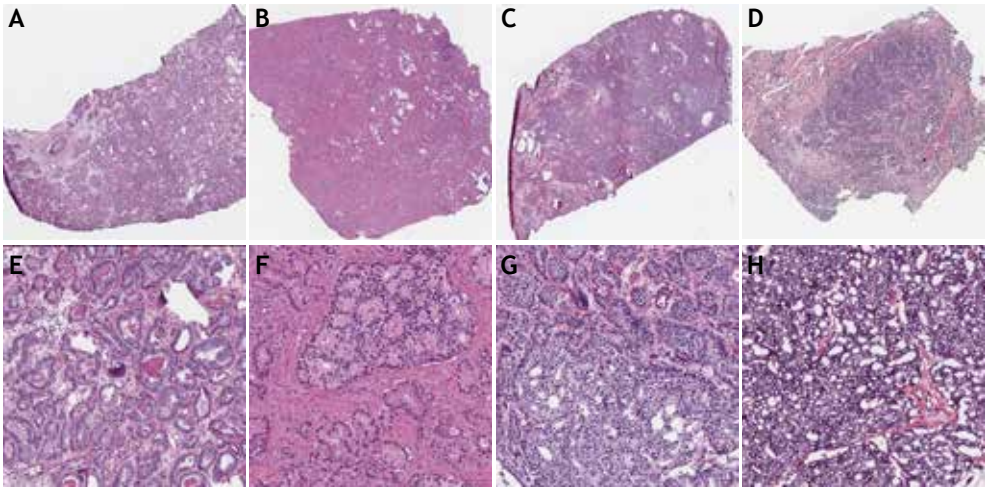


Figure 1. Representative images of reference HE slides of GS 6 (A, E) without CR/IDC, and GS 3+4=7 (B, F), 4+3=7 (C, G) and 4+4=8 (D, H) with CR/IDC growth.

Table 1. Clinical and pathological patient characteristics of the TCGA and CPC-GENE cohorts.

	Entire cohort		CR/IDC positive		CR/IDC negative	
	TCGA	CPC-GENE	TCGA	CPC-GENE	TCGA	CPC-GENE
	Mean (IQR) or n (%)					
Number	260 (100)	199 (100)	80 (31)	76 (38)	180 (69)	123 (62)
Age (years)	60 (56-66)	61 (57-66)	61 (57-66)	61 (58-66)	60 (55-70)	61 (57-64)
PSA (ng/mL)	10 (5.1-11)	7.6 (4.8-9.3)	12 (6.4-15)	8.1 (4.9-10)	9.5 (4.6-9.7)	7.3 (4.8-9.1)
GS						
3+3	96 (37)	69 (35)	0	0	96 (53)	69 (56)
3+4	78 (30)	95 (48)	27 (34)	44 (58)	51 (28)	51 (41)
4+3	39 (15)	25 (12)	22 (27)	22 (29)	17 (10)	3 (3)
8	19 (7.3)	9 (4)	17 (21)	9 (12)	2 (1)	0
9-10	28 (11)	1 (1)	14 (18)	1 (1)	14 (8)	0
pT stage						
T2	112 (43)	84 (42)	20 (25)	20 (26)	92 (51)	64 (52)
T3a	80 (31)	58 (29)	28 (35)	26 (35)	52 (29)	32 (26)
T3b	55 (21)	15 (8)	31 (39)	10 (13)	24 (13)	5 (4)
T4	4 (2)	0	1 (1)	0	3 (2)	0
Tx	9 (3)	42 (21)	0	20 (26)	9 (5)	22 (18)

GS (Gleason score); PSA (Prostate Specific Antigen)

CR/IDC is associated with genomic instability

To assess whether CR/IDC was associated with genomic instability, we calculated PGA for all patients and used a Wilcoxon-test to identify significant differences.[17,26] PGA was 3 fold ($P=1.6\cdot10^{-4}$) higher in men with CR/IDC as compared to men without (Figure 2). Exclusion of men with GS 6, who generally lack CR/IDC growth, yielded similar results with 2.2 fold ($P=3\cdot10^{-4}$) PGA increase in cases containing CR/IDC. Subgroup analysis revealed that PGA was significantly higher in samples with CR/IDC in GS 4+3=7 (2.2 fold; $P=5.3\cdot10^{-3}$), but not in GS 3+4=7 (2.1 fold; P=0.19), GS 8 (5.1 fold; P=0.57) and GS 9-10 (1.7 fold; P=0.10). Moreover, PGA scores did not differ significantly between GS 3+4=7 without CR/IDC pattern and GS 6 (1.2 fold; P=0.51). Validation within the CPC-GENE cohort revealed overall 1.7 fold higher PGA of CR/IDC positive men with GS ≥ 3+4=7 ($P=4\cdot10^{-3}$). Subgroup analysis showed 1.3 fold (P=0.02) higher PGA in GS 3+4=7 cases with CR/IDC as compared to those without. PGA scores were significantly lower in GS 6 as compared to GS 3+4=7 with CR/IDC (2.2 fold; $P=4.7\cdot10^{-7}$) than those without CR/IDC (1.6 fold; P=0.07). Since 32 out of 35 CPC-GENE patients with GS ≥ 4+3=7 had CR/IDC, statistical analysis in respective subgroups lacked statistical power.

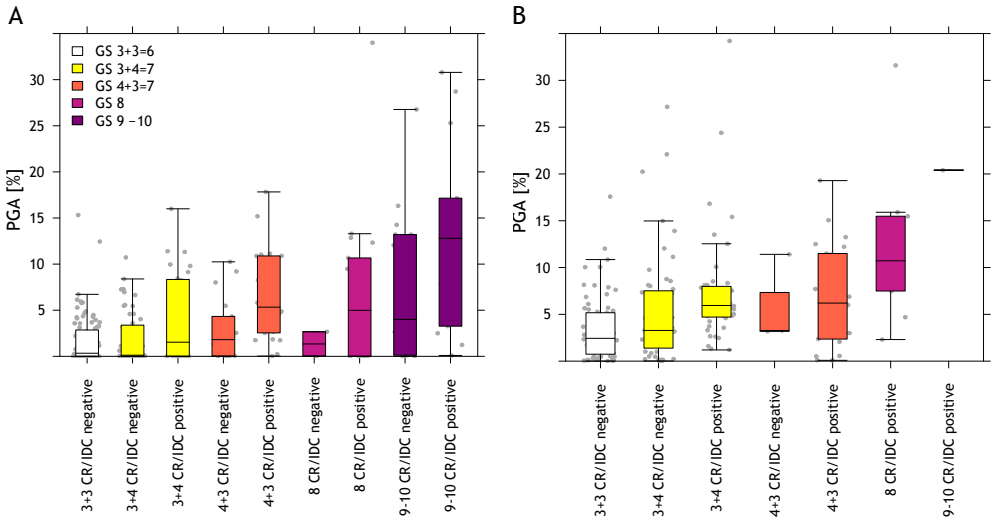
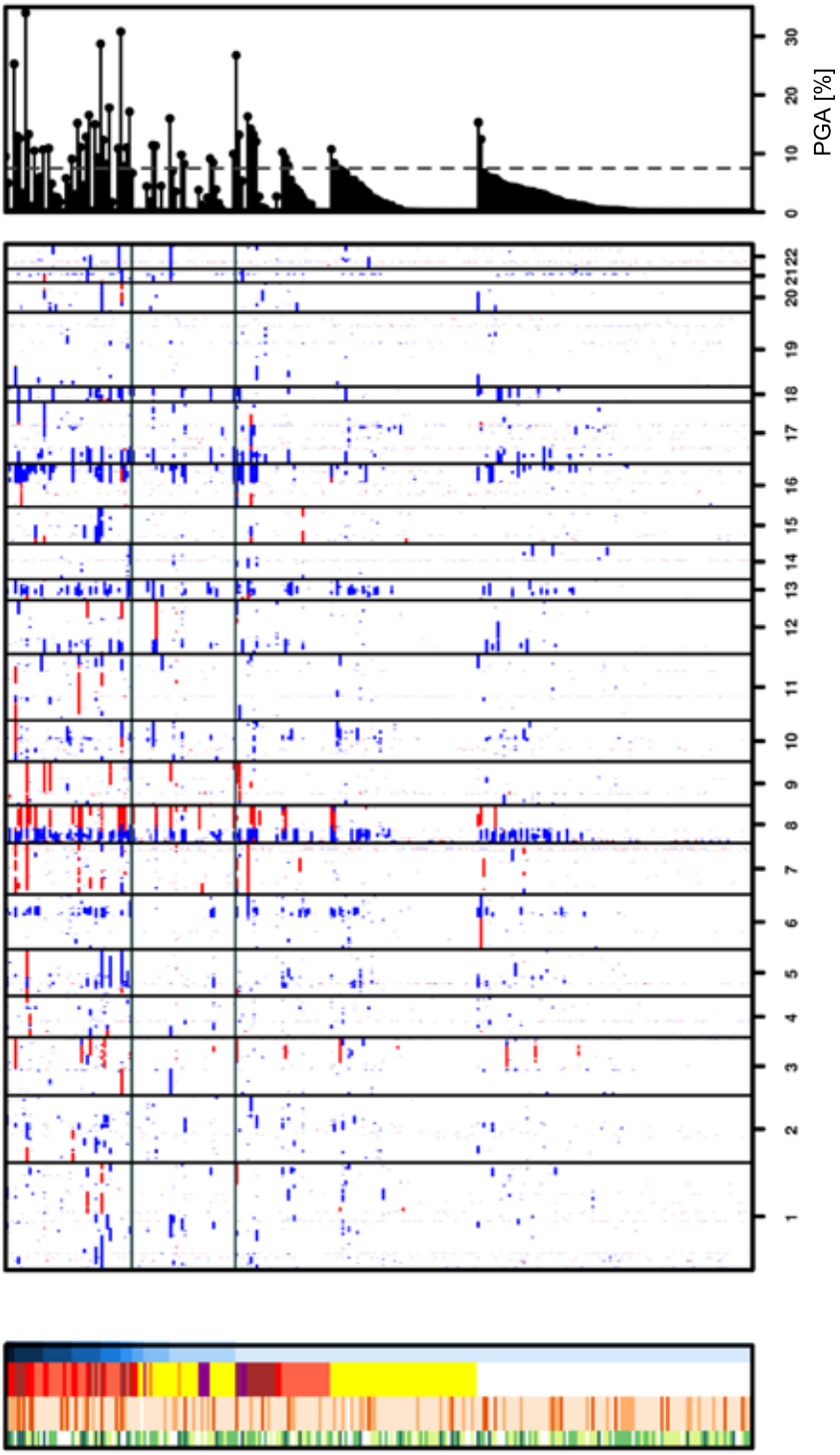


Figure 2: Boxplot of patient-wise PGA stratified by CR/IDC percentage and Gleason score in the TCGA (A) and CPC-GENE (B) cohort.

To determine whether genomic instability in CR/IDC was a global phenomenon or affected specific genomic regions, we computed PGA for individual chromosome arms utilizing deletion and amplification events independently. We found that deletions were mostly present on chromosome arms 1p, 4p, 4q, 5q, 7q, 8p, 10p, 10q, 12p, 13q, 16q, 17p, 18q and 21q in samples with CR/IDC ($P<0.05$, Supplementary Figures 2 and 3; Supplementary Table 1), while amplifications were found on chromosome 4q, 8p, 8q, 9p, 14q and 18p. Several of these chromosome arms have been linked to advanced prostate cancer.[21,33-36] Increased PGA for chromosome 4p, 8p, 10q, 12p and 16q deletions were also present in the CPC-GENE cohort ($P<0.05$, Supplementary Figures 4 and 5; Supplementary Table 1).

Somatic CNAs associated with aggressive clinical outcome are enriched in CR/IDC

To identify somatic CNAs associated with CR/IDC we applied Boschloo's exact test independently for each gene locus in GS ≥ 3+4=7 samples. We found 592 gene deletions and 366 amplifications significantly associated with CR/IDC ($q<0.05$). These events clustered in specific chromosomal regions known to be associated with aggressive disease such as deletions of 8p (*PPP2R2A*, *NKX3-1*)[37-39], 16q22 (*CDH1*)[40], 16q23 (*BCAR1*, *CTRB1*, *CTRB2*, *WWOX* and *MAF*)[15,41,42], 16q24[43], 10q23 (*PTEN*)[44,45], 17p13 and 18q21 (*CCBE1*)[46] as well as amplification of 8q24 (*MYC* and *LY6* family members)[15,47,48], Figure 3 and Supplementary Table 2).


PGA [%]
0
10
20
30
1
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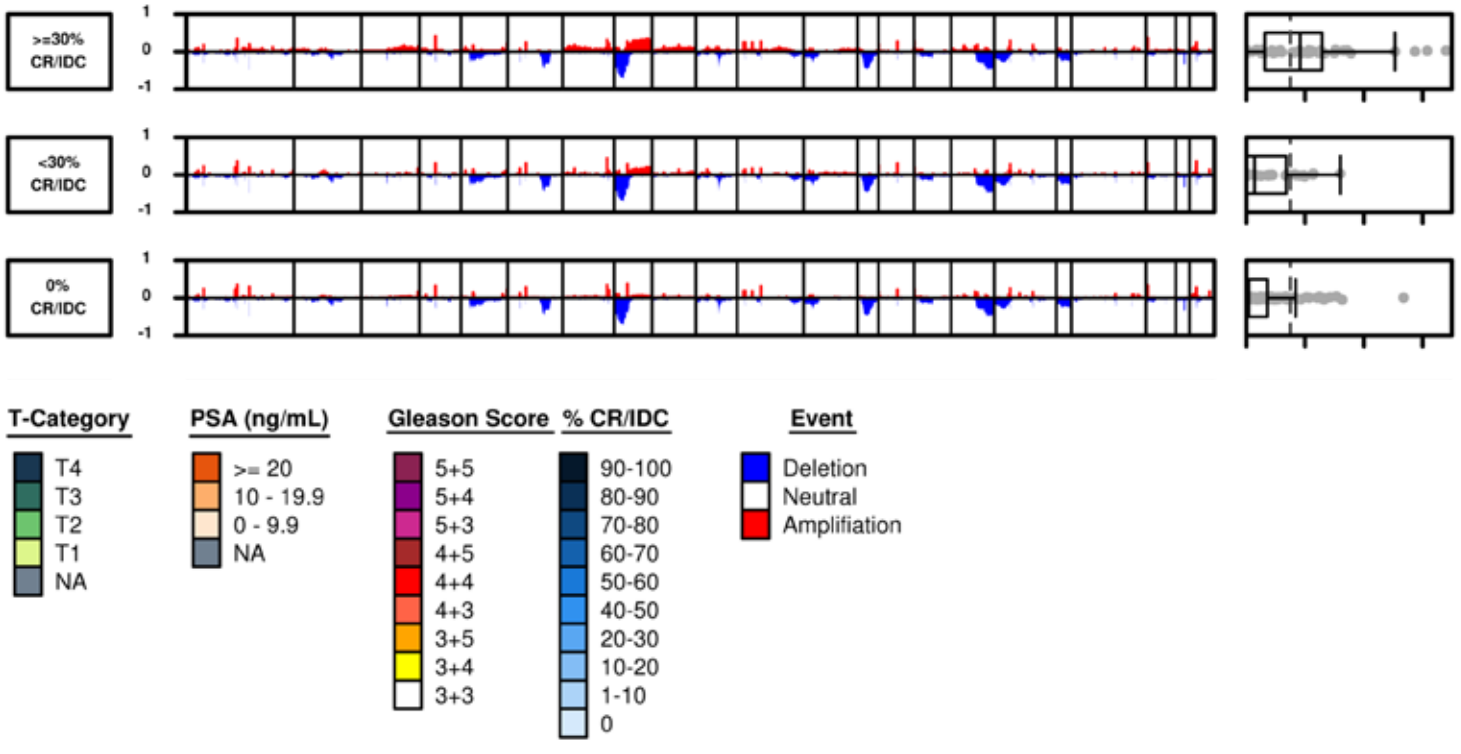

Figure 3. Overview heatmap of CNA in TCGA cohort. Clinical variables are displayed on the left, while PGA is displayed on the right. Samples are ordered by CR/IDC percentage, with two thresholds chosen to discriminate between negative (0%), intermediate (1-30%) and high (>30%) CR/IDC growth pattern.

Since it was unclear whether genomic alterations occurred specifically in CR/IDC structures or also in non-cribriform prostate cancer glands adjacent to CR/IDC, we excluded samples with <30% CR/IDC growth pattern. Comparing GS ≥ 3+4=7 men with ≥30% CR/IDC (n=44) to those without (n=84) resulted in a total of 1299 significant deletions and 369 amplifications. Additional deletions in cases with ≥30% CR/IDC included the "Down syndrome critical region" located between *ERG* and *TMPRSS2* on 21q22[49], 16q22 (*CTCF*)[50], 13q14 (*RB1*)[51,52], 17p13 (*TP53*)[53], and parts of 6q[54,55] (Supplementary Table 3). Although genetic deletions of genes located between the *TMPRSS2* promoter and *ERG* occurred more frequently in CR/IDC cases, we were unable to find a significant difference in *ERG* mRNA expression (Supplementary Figure 6). This paradoxical finding might be explained by relatively more frequent genomic translocation than deletion mechanism for *TMPRSS2:ERG* corresponding to lower genomic instability in cases without CR/IDC.[56]

A trend towards lower q-values was observed when excluding tumors with <30% CR/IDC pattern suggesting that signal strength from CR/IDC specific events was diluted in cases with low CR/IDC quantity. Subsequent analyses were all performed using CR/IDC samples with at least 30% cribriform architecture. In total 474 deleted and 328 amplified genes were validated in the CPC-GENE cohort (q<0.1), located on chromosomes 8p, 10q23, 13q22, 16q23-24, 17p13, 21q22, as well as 8q24, respectively (Supplementary Table 4 and Supplementary Figure 7). We noticed that q-values were generally lower in TCGA as compared to CPC-GENE, regardless of whether a threshold on CR/IDC was applied or not, indicating relatively lower statistical power of the latter cohort.

Since genomic instability and GS might act as confounding factors in assessing CNA events, we performed logistic regression analysis correcting for GS and PGA based on the 1668 previously identified events. A total of 779 gene deletions and 317 amplifications were independently associated with CR/IDC (q<0.1, Supplementary Table 5). Deletions were mostly located on 8p21-23, 13q14, 16q21-24 as well as 18q21-23, but also included the genomic loci containing *PTEN* (10q23)[57], *RYBP/FOXP1* (3p13)[16] and *CASP8AP2* (6q15)[58]. The *PPP2R2A/BNIP3L/PNMA2* locus (8p21)[37] featured the lowest q-value for deletions (P=0.00018, q=0.02, OR=10.2, 3.24-38), while the *MAFA/PTP4A3* locus (8q24)[59,60] did for amplifications (P=0.007, q=0.08, OR=7.77, 1.98-41.95). For CPC-GENE, logistic regression did not yield significant results after correcting for multiple comparisons, which can be attributed to lower statistical power and significant differences in pathological features.

Somatic SNVs are not main driver events for CR/IDC growth

To identify genes affected by functional SNVs we used TCGA exome sequencing data (https://tcga-data.nci.nih.gov/) of samples with GS ≥ 7, and compared 88 samples with ≥ 30% CR/IDC against 143 without. Filtering for genes that harbored SNVs in at least 5% of all samples, *FOXA1* (15% versus 5%; P=0.007), *TP53* and *SPOP* (both 19% versus 10%; P=0.035) showed significantly higher mutation rates in cases with CR/IDC compared to those without (Boschloo's exact test). Although SNV data were available for CPC-GENE samples, the number of cases, i.e. 8 with and 30 without CR/IDC was too low for statistical analysis. We did not find significant differences in overall frequency or total number of affected genes with functional SNVs (*data not shown*), indicating that SNVs are unlikely to be driver events for CR/IDC growth.

Finally, we investigated whether recently discovered DNA repair-related phenomena were linked to CR/IDC.[61,62] We utilized available computational scores for kataegis, a pattern of localized hypermutation, and chromothripsis, a catastrophic event during which single chromosome arms or entire chromosomes are rearranged and/or lost. No statistically significant differences could be identified between cases with and without CR/IDC albeit sample numbers were low (*data not shown*).

DISCUSSION

Recent studies have validated the clinical importance of both invasive cribriform and intraductal carcinoma of the prostate.[6,13,14] In the current study, we hypothesized that CR/IDC represents a morphologic substrate of genomic alterations associated with aggressive disease. We found that CR/IDC was associated with increased genomic instability showing chromosomal deletions of 3p13, 6q15, 8p21-23, 10q23, 13q14, 16q21-24, 18q21-23, and amplification of 8q24. The genetic losses and amplifications included several genes related to aggressive prostate cancer, such as loss of *PTEN*, *RB1*, *TP53* and amplification of *MYC*. These findings altogether support our hypothesis that CR/IDC is a specific morphologic substrate of genomic alterations associated with aggressive disease.

Our study is in line with previous studies on genetic abnormalities related to CR/IDC growth. Dawkins *et al*. and Bettendorf *et al*. observed more frequently loss of heterozygosity (LOH) in IDC than in the invasive prostate cancer component.[63,64] Qian *et al*. showed gain of chromosomes 7, 12, and Y, loss of chromosome 8, and amplification of *c-MYC* in cribriform cancer compared to other Gleason grade 3 and 4 patterns.[65] In a meta-analysis on recurrent CNAs, Williams *et al*.[34] compared 568 primary prostate cancer tumor samples from 8 previous studies[16,19,20,66-70] with 115 metastatic prostate cancer samples from 5 previous studies.[16,22,68,71,72] Strikingly, the prevalence of recurrent CNAs in metastatic prostate cancers corresponded with several of the CNAs that were enriched in CR/IDC growth, such as *PTEN* and *NKX3-1*. Recently, Chua *et al*. studied differences in RNA expression in prostate cancer with and without CR/IDC. They found that the long non-coding RNA SChLAP1, which has been associated with tumor progression, was significantly higher in CR/IDC, and that CR/IDC growth was associated with hypoxia.[73-75] Together these findings further support a strong relation of CR/IDC with molecular tumor progression. On the other hand, we did not find a statistically significant difference between CR/IDC-negative GS 3+4=7 and GS 6 cases, which further supports the question whether it is clinically relevant to distinguish CR/IDC-negative GS 3+4=7 from GS 6 prostate cancer.

Although prostate cancer with CR/IDC showed increased genomic instability, it is not yet clear to what extent these molecular alterations are exclusively present in CR/IDC tumor glands or whether these alterations can also be found in surrounding non-cribriform tumor glands. Using RNA *in situ* hybridization, we previously found that SChLAP1 was not only over-expressed in CR/IDC structures but also in adjacent non-cribriform cancer glands suggesting that it represents a field effect during tumor progression and not a specific characteristic of CR/IDC growth.[73,76] In our study, CR/IDC was more frequently present in cases with higher GS. To exclude that genomic alterations were merely relating to higher GS

and not to CR/IDC *per se*, we performed PGA subgroup analysis and logistic regression for CNAs, which indeed revealed an independent associated with CR/IDC in the TCGA cohort. Further comparisons of microdissected growth patterns within individual patients are mandatory to determine what events are specific for CR/IDC and which represent general effects of progression.

Elucidation of the molecular alterations associated to CR/IDC is not only of interest for molecular-biology, but might also have future impact for prostate cancer diagnosis and management. Prostate biopsies only sample a limited volume of the entire tumor and might be false-negative for CR/IDC due to sampling artifact. Since IDC represents an extensive proliferation of neoplastic cells within pre-existent acini, which connect with the urethra, we postulate that these cells and/or their DNA can be shed into urine. Identification of molecular alterations associated with CR/IDC in voided urine could form the base of non-invasive tests for detection of aggressive CR/IDC.

The current study has several limitations. While we set out to validate our findings in an independent cohort, we noticed that many events originally found in the TCGA cohort could not be confirmed in the CPC-GENE dataset. This may be explained by differences in cohort composition, since the TCGA was enriched for tumors with adverse pathologic features. In addition, the statistical power of the CPC-GENE cohort was lower than of the TCGA, as its study population was smaller, included samples with lower and more variable tumor percentage, and was strongly enriched for CR/IDC in GS 8-10. Nevertheless, both datasets independently revealed the association of CR/IDC with increased genomic instability and the deletions of various specific genomic regions and genes. Furthermore, tumor heterogeneity and sampling artifacts may have also influenced the outcome of this study, as our current data was based on DNA derived from a freshly frozen section per patient. Hence, there may have been, for instance, CR/IDC growth in an adjacent region that was not sampled for genomic analysis that may have been detected due to a field effect. This might be the cause of the relatively small effect sizes in the current study. Lastly, we did not independently analyze CR/IDC growth in relation to adjacent tumor glands using, for instance, laser-capture microdissection or *in situ* hybridization.

CONCLUSION

We found that pathologic CR/IDC growth pattern is associated genomic instability including deletions of 8p, 10q23, 13q22, 16q22-24, 17p13 and 21q22, as well as smaller 8q24 amplification. These results indicate that CR/IDC is a histopathological substrate of molecular tumor progression and presents a rationale for its aggressive clinical behavior.

SUPPLEMENTARY FILES

Accessible via https://bmccancer.biomedcentral.com

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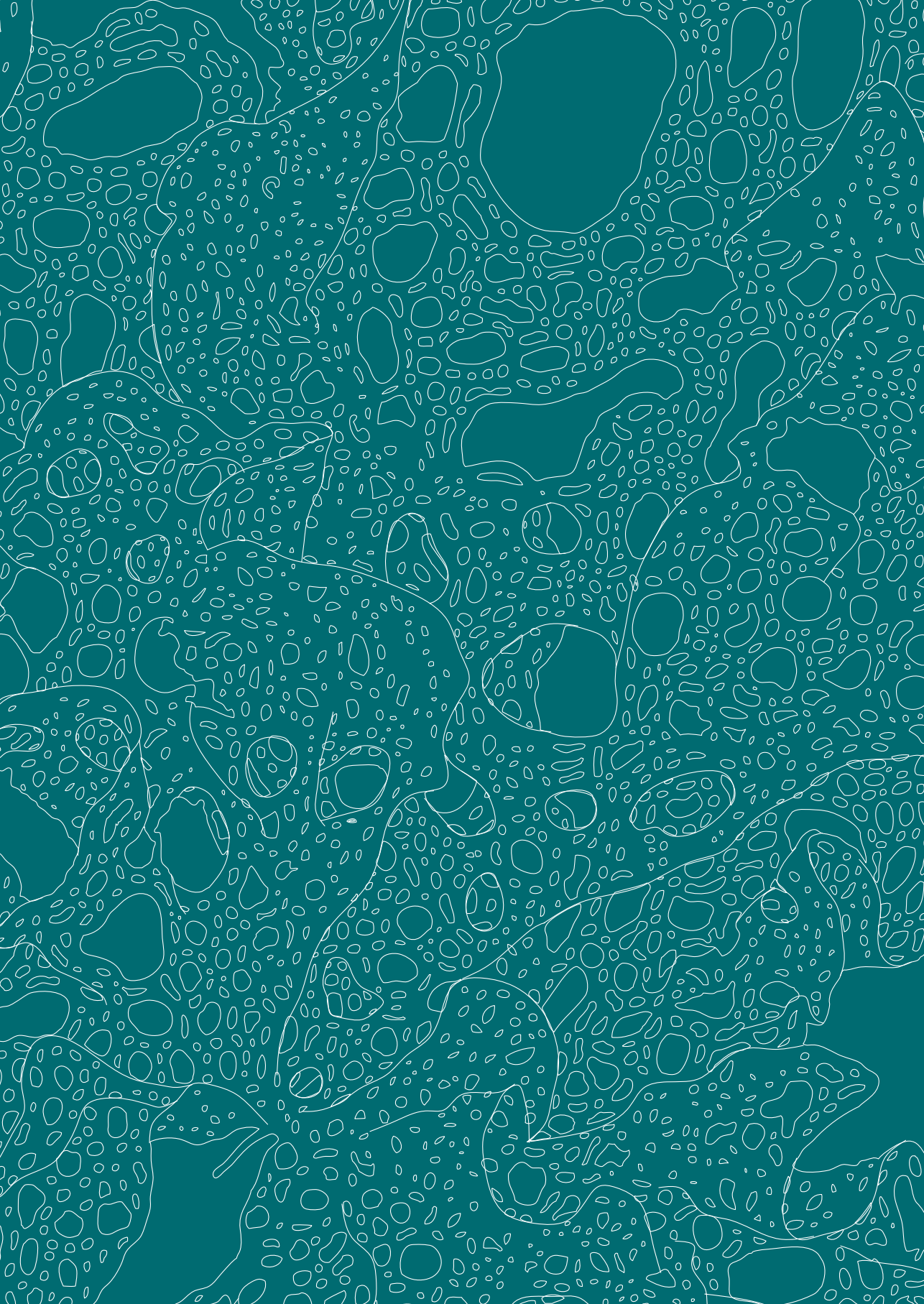

DISCUSSION

The objective of this thesis was to study the role of cribriform growth in prostate cancer with respect to diagnosis, prognosis and molecular pathology. In this chapter, I will first summarize the main findings and review their interpretation in the context of the current knowledge. I will then provide a discussion on methodological considerations and clinical applications. Lastly, I will discuss recommendations for future research.

REVIEW AND INTERPRETATION OF MAIN FINDINGS

Is Gleason score 3+3=6 really malignant?

One of the main questions in this thesis is whether pure Gleason score 3+3=6 tumors have the potential to metastasize. Previous studies on Gleason score 6 patients have reported negligible rates of biochemical recurrence after radical prostatectomy or salvation radiotherapy.[1-3] Hernandez *et al.*, for instance, showed that patients with organ-confined Gleason score ≤6 prostate cancer do not develop post-operative metastases nor die from prostate cancer.[3] In a more recent analysis containing >14 000 Ross *et al.* did not find any lymph node metastases in men with modified Gleason score ≤6 prostate cancer during follow-up.[4] Our study is in line with these findings, and additionally shows that distant metastasis and disease-specific death do not occur in non-organ confined Gleason score ≤6 prostate cancer either.[5] We therefore hypothesize that these tumors do not have the biologic ability to metastasize or cause disease-specific mortality.

Previous autopsy studies have already provided considerable evidence that a substantial number of prostate cancers within the population exist that do not result in clinical symptoms or death. These, for instance, reported incidental prostate cancer prevalence between 30 and 40% in men older than 50 years.[6-8] Of these incidentally detected cancers, at least 70% (cystoprostatectomy series) and 80% (autopsy series) showed presence of Gleason score 6 prostate cancer.[6-10] These data support the argument of considering Gleason score 6 as a benign lesion. Additionally, the Gleason grading modification in 2005 exceedingly limited the Gleason pattern 3 definition. As a result, men diagnosed with Gleason score 6 prostate cancer today are likely to have less aggressive tumors than those with a classical Gleason score 6 prostate cancer diagnosed before 2005.[11]

The fact that Gleason score 6 prostate cancers have excellent outcome, raises the question by urologists whether these tumors are cancer at all. There are, however, several arguments against diagnosing Gleason score 6 as a benign lesion. Our study, for instance, showed that Gleason score 6 tumors are able to expand through the prostatic capsule and to invade the seminal vesicles, indicating local aggressive behavior similar to other malignancies. Prostate cancer glands also show loss of the basal cell layer microscopically, indicating invasion in the surrounding tissue. Gleason grade 3 glands additionally share molecular alterations associated with Gleason grade 4 glands, such as the *TMPRSS2-ERG* gene fusions.[12,13] Furthermore, it is important to realize that studies showing excellent outcome of Gleason score 6 prostate cancer had been performed using radical prostatectomy specimens. This means that the entire tissue was available for histopathological analysis and does not include presence of high-grade components. Since considerable sampling

error exists in diagnostic biopsies, these results cannot be directly translated to diagnostic biopsies. Like many solid tumors, prostate cancer is a heterogeneous disease and susceptible to biopsy sampling error. The biopsy Gleason score can underestimate the actual grade due to missing significant tumor components. In a recent study containing 7643 radical prostatectomies with corresponding needle biopsies, Epstein *et al*. reported that 36% of cases (1841/ 5071) were upgraded from a needle-biopsy Gleason score 6 to a higher grade at radical prostatectomy.[14] Based on large active surveillance studies in men with Gleason score 6 on biopsy, up to 33% of the patients still need therapeutic intervention primarily due to Gleason score upgrading.[15-20] At this moment, it is however impossible for the pathologist to determine which Gleason grade 3 glands in a biopsy specimen represent a pure Gleason score 6 tumor or a component of higher-grade tumor in which the high-grade component is not present in the respective tissue. Factors that have been associated with upgrading are serum PSA levels, clinical stage and tumor volume on biopsy.[14] In addition, other ancillary tests that are still under investigation, such as mpMRI and molecular testing, show promising results.[21-25] These tests could be, for instance, useful in identifying alterations associated with either low-risk or high-risk disease in Gleason score 6 patients.

Although we did not report any deaths attributed to cancer in men with modified Gleason score 6 prostate cancer at radical prostatectomy, we and Ross *et al*. originally found several cases of metastatic disease in classic Gleason score 6 patients.[4,5] Interestingly, microscopic review of their histology demonstrated presence of Gleason grade 4 pattern elements in all cases. These patients were treated and graded in the era before the 2005 Gleason grading modification when these patterns were still considered as a grade 3 pattern.[11] An important observation from these pathology reviews, which laid the foundation for this thesis was that the majority of the classic Gleason score 6 patients who had developed metastatic disease showed presence of cribriform tumor glands in their specimen.[4]

Cribriform prostate cancer

Our group has previously found that presence of cribriform growth in radical prostatectomy specimens is a major predictive factor for distant metastasis and disease-specific death of prostate cancer in Gleason score 7 patients. In fact, cribriform growth was the strongest predictor of both adverse clinical events after surgical treatment in multivariable analysis, adjusted for other relevant clinico-pathologic variables, such as age, PSA, Gleason score and pT stage.[26] In the past years several other groups using different patient cohorts and various clinical endpoints additionally validated the association of

cribriform growth with adverse outcome.[27-33] We subsequently validated the independent prognostic value of cribriform growth in diagnostic needle biopsies using strong clinical endpoints. Importantly, we found that patients with Gleason score 3+4=7 without cribriform growth on diagnostic biopsy have similar patient outcomes as those with Gleason score 6, implying these patients may be potential candidates for active surveillance as well.[34,35]

The cribriform pattern shows good interobserver reproducibility among pathologists, while patterns such as fused and ill-formed Gleason grade 4 are poorly reproducible.[36] Another study showed that the percentage of fused and ill-formed glands was inversely correlated with agreement among pathologists, whereas the cribriform pattern had no significant correlation with interobserver variability.[37] This supports the hypothesis that cribriform growth might be a valuable additional parameter in selecting patients for active surveillance. In our final study, we demonstrated that cribriform prostate cancer is associated with increased genomic instability showing chromosomal deletions of 3p13, 6q15, 8p21-23, 10q23, 13q14, 16q21-24, 18q21-23, and amplification of 8q24.[38] The genetic losses and amplifications included several genes related to aggressive prostate cancer such as loss of *PTEN*, *RB1*, *TP53* and amplification of *MYC*. Our study is in line with previous studies on genetic abnormalities related to cribriform and/or intraductal carcinoma using comparative genomic hybridization. Two studies observed more frequently loss of heterozygosity (LOH) in IDC than in the invasive prostate cancer component.[39,40] Qian *et al*. showed gain of chromosomes 7, 12, and Y, loss of chromosome 8, and amplification of *c-MYC* in cribriform cancer compared to other Gleason grade 3 and 4 patterns.[41] The latter three studies, however, contained small sample sizes, while our current study included a large number of patients.[38] In a meta-analysis on recurrent CNAs, Williams *et al*. compared 568 primary prostate cancer tumor samples from 8 previous studies with 115 metastatic prostate cancer samples from 5 studies.[42] Remarkably, the prevalence of recurrent CNAs in metastatic prostate cancers corresponded with the CNAs found enriched in cribriform prostate cancer, such as *PTEN* and *NKX3-1*. More recently, using break-points regions to infer phylogenetic relationships, Lindberg *et al*. showed that the clone closely related to the distant metastasis was found in intraductal carcinoma that had cribriform architecture.[43] Altogether, these findings further support a strong association of cribriform growth with molecular tumor progression. Vice versa, we did not find a statistically significant difference in genetic abnormalities between Gleason score 3+4=7 without cribriform growth and Gleason score 6 cases, supporting the notion whether it is clinically relevant to distinguish cribriform-negative Gleason score 3+4=7 from Gleason score 3+3=6.

What about the other grade 4 patterns?

After the ISUP consensus conference in 2005, ill-formed (or poorly formed) glands were considered a Gleason grade 4 pattern.[11] The authors additionally recommended that high-grade tumor of any quantity on needle biopsy should be included within the Gleason score. Thus, a needle biopsy that is involved by cancer with 98% Gleason pattern 3 and 2% Gleason pattern 4 would be diagnosed as Gleason score 3+4=7. The Gleason score system modification in 2005 led to a significant grade inflation, i.e. a decline in reported incidence of Gleason score 6 tumors and relative increase of Gleason score 7 tumors. The modification resulted in better clinical outcomes in both patient populations, a statistical artifact also known as the Will-Rogers phenomenon.[44,45] Patients with Gleason score 6 prostate cancer are considered candidates for active surveillance, whereas patients with Gleason score 7 generally undergo therapeutic intervention.[46] Others and we have shown that the ill-formed pattern has a considerable intraobserver and interobserver variability among pathologists.[36,47-52] This poorly reproducible pathologic variable is nonetheless an important clinical decision point for many patients. Patients with Gleason score 6 prostate cancer are candidates for active surveillance, whereas patients with Gleason score 7 generally undergo therapeutic intervention.[46] As a matter of fact, no studies to date have specifically validated the adverse prognostic value of the ill-formed pattern and its role in active surveillance enrolment of patients with prostate cancer. Zhou *et al*. recently suggested that adjacent tumor glands play an important role in decision-making in cases showing ambiguous ill-formed patterns.[52] The authors recommend that >10 poorly formed glands not immediately adjacent to other well-formed glands should be considered to represent ill-formed Gleason pattern 4. In contrast, poorly formed glands that are intermixed with well-formed glands, or ≤5 poorly formed glands, regardless of their location, should be diagnostic features arguing against Gleason pattern 4. Although such criteria seem reasonable, they are – like many previous studies on the distinction of well-formed pattern 3 glands versus ill-formed pattern 4 glands – not based on clinical outcome data. Secondly, and perhaps more importantly, as demonstrated by Labov's linguistic work, endeavors to set a classification threshold for categories along a continuum leads to significant problems with category reproducibility.[53] The ill-formed pattern is poorly reproducible and we agree with McKenney *et al*. that the specific histologic assessment of "ill-formed glands" will never reach a high level of diagnostic reproducibility for any group of pathologists, regardless of more specific criteria or increased education.[32] We therefore believe that the ill-formed pattern itself should not be a criterion to exclude a patient from active surveillance, as the higher Gleason score most likely reflects a change in grading practice rather than tumor biology.

In 2009, Lotan *et al.* were the first to our knowledge to publish a paper on grading prostate cancer with glomeruloid features.[54] In this study the authors claimed that the glomeruloid pattern is strongly associated with high-grade prostate cancer on the same biopsy core (36/45, 80%). Based on the observation that in several cases a transition could be seen among small glomerulations, large glomeruloid structures, and cribriform pattern 4 cancer, the authors additionally suggest that glomerulations represent an early stage of invasive cribriform cancer and are best graded as Gleason pattern 4. These observations lay the foundation for the current ISUP recommendations, which recommend that glomeruloid glands should be assigned a Gleason pattern 4, regardless of morphology.[54,55] No clinical outcome data was, however, available from the study by Lotan *et al*.[54] Although their suggestion regarding grading seems plausible and pragmatic too, others and we could not find an association between glomeruloid and cribriform glands or high-grade cancer.[26,33] Moreover, both our studies found that presence of glomeruloid glands is independently associated with a better outcome of Gleason score 7 prostate cancer in multivariable analyses, which contradicts the idea that glomeruloid glands represent a precursor lesion of an aggressive cancer type. McKenney *et al.* could also not find an association between glomeruloid glands and outcome.[32] We believe that the smaller glomerulations surrounded by well-formed pattern 3 glands are more likely to show more indolent behavior than those transitioning to large glomerulations and/or cribriform glands. Interestingly, in our interobserver reproducibility study on Gleason grade 4 patterns we found that there is good interobserver reproducibility of small glomeruloid glands, but less in large glomeruloid glands as half of the observers considered these cribriform.[36] Similar to the semantics in well-formed glands and ill-formed glands, there seems be a continuum in morphology of large glomeruloid and cribriform glands. The biology of glomeruloid glands, let alone their pathological meaning, remains unknown.

Intraductal carcinoma of the prostate

In recent years the clinical significance of intraductal carcinoma of the prostate – a morphological mimicker of invasive cribriform carcinoma – has been acknowledged. The current concept is that it represents divergent differentiation of a common precursor that either spreads invasively or via pre-existing ducts.[56] Although not included in the Gleason grading system, intraductal carcinoma has been associated with Gleason grade 4 and 5 patterns, advanced tumor stage, biochemical recurrence and distant metastasis.[57-62] Although invasive cribriform carcinoma and intraductal carcinoma are strictly speaking two different pathologic entities, they morphologically mimic each other closely and it is

possible they relate and exist on a pathological and biological continuum.[63,64] In our studies we noticed in the majority of cases that both entities co-exist in the same tumor.[26,34] The current concept is that intraductal carcinoma represents spread of high-grade prostate cancer into pre-existing ducts using these natural passages as low-resistance highways of rapid growth.[39,63,65] We found that intraductal carcinoma and invasive cribriform glands are often present together in prostate cancer specimens, raising the question whether invasive cribriform glands could possibly represent invasion of intraductal carcinoma into surrounding tumor glands. Also, the lack of basal cells is not pathognomonic of invasive cribriform cancer as basal cells can be scattered and left unsampled in the slide. To date, little is known about how, for instance, intraductal carcinoma transitions to invasive cribriform cancer on a molecular and three-dimensional level. Are gland sizes or specific stromal-epithelial interactions creating a complex anastomosing network of tumor glands of pathological significance? In fact, we do not know what drives the formation of cribriform tumor glands. Although we find several genetic abnormalities associated with cribriform growth in prostate cancer, it remains unclear how the phenotype and genotype interact.

METHODOLOGICAL CONSIDERATIONS

Study design

A limitation of our Gleason score 3+3=6 mortality study was that not all radical prostatectomy specimens were pathologically reviewed and scored according to the modified Gleason score.[5] Recently, Dong *et al*. re-graded 806 radical prostatectomies with Gleason score 3+3=6 and 3+4=7 prostate cancer according to the modified Gleason grading system and report an upgrade of 34% from classical Gleason score 3+3=6 prostate cancer to modified Gleason score 7 or 8 at radical prostatectomy, but not a single case of Gleason score ≥7 was downgraded to a ≤6 at radical prostatectomy.[66] For this practical reason we only revised the classical Gleason score 3+3=6 cases with adverse patient outcome. Although we claim that Gleason score 3+3=6 patients do not die from their disease, all patients had received treatment. One could argue that these patients had been cured by their therapy and therefore had not developed metastatic disease.

Another limitation from patient-outcome studies in this thesis is the retrospective design. For instance, the original ERSPC protocol included sextant biopsies, while current biopsy schemes are more extensive and increasingly MRI targeted aiming to reduce the chance of sampling artifact. In addition, there is a difference in treatment modalities compared with the 1990s. Low-risk patients studied in this thesis had mostly received active treatment, while today active surveillance would have been an acceptable strategy for them. Nevertheless, studying a variable's prognostic impact with disease-specific death as an endpoint in prostate cancer demands for a long-term follow-up. Further research in a prospective setting is needed to validate the prognostic value of Gleason grade 4 patterns in contemporary protocol-based active surveillance protocols.

Interobserver variability

When pathologists look at a tumor under the microscope, they essentially look at a snapshot of a process that continuously grows and changes over time. This continuum makes it challenging for pathologists to set reproducible classification thresholds and offers an explanation why the Gleason grading system in prostate cancer remains subjective despite various international consensus meetings to define criteria. Although variability in pathological grading does not always affect clinical-decision making, some thresholds have major clinical impact. In prostate cancer, for instance, the discrimination of Gleason score 3+4=7 from 3+3=6 has clinical consequences. For our studies we scored many histological slides for various growth patterns, but cannot be certain that our judgment has been entirely

correct in all of the cases. If other pathologists, or even we, would repeat this job there will be most likely variation in pattern classification, particularly in the identification ill-formed and fused glands. The fact that our findings indicate that Gleason score 3+4=7 patients with ill-formed glands have similar outcomes to those with Gleason score 3+3=6 further supports the poor reproducibility of these glands. We therefore believe that the ill-formed pattern itself should not be a criterion to exclude a patient from active surveillance, as the higher Gleason score most likely reflects a change in grading practice rather than tumor biology.

Percentage Gleason grade 4

Recent literature has suggested that quantifying the percentage of Gleason grade 4 may be a more useful tool for risk prediction.[67-69] Although most Gleason score 3+4=7 disease are recommended to undergo active treatment, selected low-volume Gleason score 3+4=7 patients could be considered for active surveillance. Recent guidelines recommend that patients with low-volume Gleason score 3+4=7 should only be considered for active surveillance if there is focal presence of Gleason grade 4, i.e. accounting for 10% of the total tumor volume.[70] Based on our study, higher Gleason grade 4 percentages are often associated with presence of cribriform tumor glands.[71] Since in our study percentage Gleason grade 4 was inferior to presence of cribriform growth with regard to predicting patient outcome in a multivariable model, the quantifying approach does, to our opinion, not really offer a solution. Determining the Gleason grade 4 percentage greatly depends on core length and interobserver variability of high-grade patterns that are poorly reproducible. Although quantification of Gleason grade 4 percentage seems an objective tool, it is more likely a semblance of precision. We therefore endorse a more practical approach by establishing the presence of cribriform tumor glands, which is a reproducible qualitative feature instead of quantification of inherently imprecise quantification of growth patterns.

Bioinformatics

While analyzing the two different patient cohorts, we noticed that a high number of copy number events detected in TCGA could also be validated in CPC-GENE when inspecting them individually, which was no longer the case after correcting for the multiple comparisons problem, implicating a lower statistical power for CPC-GENE due to the smaller sample size. Furthermore, both datasets differed in terms of the utilized array platform as well as Gleason score distribution, with TCGA featuring higher stage samples while CPC-GENE focused on lower to intermediate grade prostate carcinomas. The two cohorts

also showed significant differences in tumor percentage, which might result in a lower signal-to-noise ratio in CPC-GENE compared to TCGA. Here, signal can be understood as the probability of being able to detect CNAs by measuring the amount of DNA. Since each measurement is taken from a mixture of cells, deviations from the baseline of two copies can only be detected if sufficient cellular material contains the altered DNA sequence and therefore causes a signal increase (amplification) or decrease (deletion) that exceeds the background noise. Therefore, with a higher number of non-cancerous cells present in each sample, the chance to observe cribriform-specific events diminishes as the global average converges to the baseline of two copies. In combination, these differences might have influenced our ability to robustly identify CNA events in the CPC-GENE cohort in a similar manner as for TCGA.

Ideally, the discovery and validation cohort should feature identical characteristics and should be derived from the same background population, such as when randomly sub-dividing a large dataset into training and testing set for cross-validation. Unfortunately, despite the enormous efforts undertaken in both studies, none of them features enough patient samples to support the required number of samples in each sub-group analyzed in our study (with one degree of freedom, ~32 samples are required per group to detect differences with an intermediate-strength effect size of 0.5 at a significance level of 0.05 with statistical power of 80%). Since in practice one has to account for additional differences due to heterogeneity of the disease, this number can easily increase to 50 and more samples for smaller effect sizes that result from CNAs being absent in subsets of patients.

Since both datasets separately suffer from a lack of samples, an alternative option would be to merge them into a larger dataset while accounting for batch effects, however, given the number of potential sources of variance (tissue sampling, DNA extraction, array platform, etc.), we chose to treat both cohorts independently and to focus on recurring events. To gain more statistical power and alleviate some of the negative effects mentioned, we reduced the number of tests by binning genes with matching copy number profiles and only performing one representative comparison for each group of genes. We also chose to use Boschloo's exact test, a more powerful version of the classical Fisher's exact test, for our analyses. In order to do so discrete calls for amplifications and deletions were required, prohibiting us from distinguishing heterozygous and homozygous deletions as well as differing number of copies for amplified genes. Moreover, the chosen thresholds for calling amplifications and deletions can influence downstream analyses by over- or under-calling events. To avoid such issues as much as possible, our thresholds were chosen after close inspection of the distributions of log2 copy numbers of both datasets. Nevertheless, further improvements of the analytical pipeline might be achieved by comparing raw log2

copy number values instead of discrete calls, which could be implemented using ANOVA-like tests. This could allow an improved sub-grouping of samples, as a differentiation between hetero- and homozygous samples would be possible.

With respect to the validity and reproducibility of our findings, despite the large discrepancies between both cohorts, we showed that cribriform-positive status can be consistently correlated with increased genomic instability and specific CNA events. Moreover, our multivariable analysis in TCGA provided evidence that cribriform growth can be an independent predictor of outcome even when correcting for Gleason score, and an independent validation using an orthogonal technique will be needed to clarify whether cribriform growth indeed harbors distinct genomic aberrations compared to surrounding tissues.

CLINICAL APPLICATIONS

Correlation with radiology

As multiparametric magnetic resonance imaging (mpMRI) of the prostate progresses, better correlation with histology could possibly lead to pre-biopsy identification of cribriform tumor glands and at the same time used as a triage test to avoid unnecessary biopsies. To date, only two recently published studies have looked into the histologic correlation between MRI findings and cribriform growth, but they show conflicting results.[72,73] However, as more research groups are becoming aware of the potential clinical relevance of cribriform prostate cancer, we expect that future MRI-correlation studies will give a better view on the pathologic-radiologic correlation.

Risk prediction

Previous studies have shown that the risk calculator number 3 (RC3) of the European Randomized Study of Screening for Prostate Cancer (ERSPC; www.erspc.org) based on the Rotterdam cohort is an adequate risk-stratifying tool in men before prostate biopsy.[74-76] The RC3 uses pre-biopsy information such as PSA, digital rectal examination outcome and prostate volume to predict the probability of a biopsy-detectable prostate cancer and/or presence of Gleason score 3+4=7 cancer or higher. The current definition of clinically significant prostate cancer is, however, largely based on the presence of any amount of grade 4. We therefore suggest to include cribriform growth in a risk calculator as the parameter for clinically significant Gleason score 3+4=7 prostate cancer. Presence of other grade 4 patterns would then be acceptable. In a recent study we aimed to improve the RC3 by inclusion of cribriform pattern in the definition of clinically significant prostate cancer. Using cribriform-specific information we found that 10% of the patients that were initially considered of having low-risk prostate cancer were upgraded to high-risk prostate cancer, and vice versa 33% were downgraded.[77] Incorporating cribriform-specific information could aid in the decision whether or not to do an MRI or biopsy. To date, Gleason score 7 has been used as an important clinical endpoint in many studies, and sometimes even defined as ‘high-risk disease’, while it appears to be a rather subjective variable with doubtful clinical relevance. We therefore recommend including presence of cribriform growth in studies using Gleason score 7 cancer as an outcome measure, since this variable seems more reproducible and clinically relevant.

Identifying therapeutic targets

As described previously, cribriform prostate cancer is associated with an adverse outcome. Prognostic value does, however, not equal predictive value. In fact, we know little about the role of cribriform growth as a predictive marker for response to androgen-deprivation therapy or chemotherapy. Also, little is known about how cribriform tumors respond to radiotherapy. Interestingly, one recent study using patient-derived xenografts of patients with advanced prostate cancer has demonstrated that intraductal carcinoma lesions are more likely to persist after androgen deprivation therapy.[78] Further understanding of the biology of cribriform growth may translate into preclinical studies to find effective therapeutic drugs for recurrent or metastatic cribriform prostate cancer.

SUGGESTIONS FOR FUTURE RESEARCH

The work described in this thesis has put cribriform growth forward as a relevant biomarker in prostate cancer. Our findings need, however, to be validated and further evaluated in longitudinal investigations. Our work has additionally generated many new questions that merit consideration in future research.

Comprehensive genomic analysis of cribriform prostate cancer

Our study on copy number variations and genomic instability in cribriform prostate cancer is just a mere start to what can be explored.[38] Further and more comprehensive studies including, for instance, transcriptomic and epigenomic data are needed to acquire a better understanding of cribriform growth in prostate cancer. *In situ* hybridization experiments could further elucidate whether specific copy number variations or differentially expressed genes are limited to the cribriform tumor glands or also seen in the surrounding tumor glands. Molecular studies could also give more insight into the differences between invasive and intraductal cribriform prostate cancer.

Biology of cribriform morphology

Cribriform morphology is not only seen in prostate adenocarcinoma, but in many other adenocarcinomas of various organs. By studying adenocarcinomas with cribriform morphology from different organs, we might find a common genetic denominator. Cribriform adenocarcinomas of the lung, stomach and colon are also associated with an adverse outcome, while cribriform adenocarcinomas of the breast and thyroid have an excellent outcome.[79-84] According to the molecular classification of breast cancer, invasive cribriform carcinoma is mainly of the luminal A-type, as estrogen and progesteron receptors are positively immunoexpressed, while negative for increased expression and/or amplification of Her2 receptor.[80] In lung cancer, Mackinnon *et al*. was unable to find a specific molecular signature for cribriform predominant carcinomas, whereas Warth *et al*. showed high rates of *KRAS* mutations, but none in *EGFR*.[82,85] In micro-satellite unstable colon cancers, Kim *et al*. found an association between adverse outcome and cribriform morphology.[83] In thyroid cancer, both the prognosis as well as the molecular alterations (*i.e.*, presence of *RET*/*PTC* translocation, and no *BRAF* mutations) are similar to those discovered in conventional papillary thyroid carcinoma.[81] Based on these findings, none of these cribriform tumors share a common genetic denominator, but they show aberrations seen in other adenocarcinoma

subtypes in the same organ. However, data containing comprehensive description of genomic, transcriptomic and epigenomic changes in numerous different tumor types and/or subtypes are now increasingly available online, some of which also containing digital histological slides. Similar to what we have done in our study, all adenocarcinomas with cribriform morphology could easily be scored by pathologists and compared to each other.

Urine-based molecular diagnostics

No matter how many prostate needle biopsies are taken, there is always a risk of sampling error. If we can identify specific genetics events for cribriform prostate cancer, we could intercept the biopsy sampling error by analyzing the patient's urine. The prostate glands drain in the urethra prostatica. We therefore hypothesize that genetic material from cribriform prostate cancer that has been spread in preexisting ducts (intraductal carcinoma) can be more easily detected in voided urine than the genetic material from invasive tumor glands. From the latter we do not know if and how they are connected to the urethra prostatica. Voided urine is increasingly being used urological cancer diagnostics by measuring cancer-associated proteins, RNA transcripts, and methylation. Sample collection of urine is non-invasive and patient friendly. Although using copy number variation analysis may be suboptimal due to contamination with normal diploid cells from the urothelium and benign prostate epithelium, further studies on transcriptomics and epigenomics might reveal interesting candidate genes that can be more easily detected in urine.

Three-dimensional imaging

Histology is two-dimensional, while tumors grow three-dimensionally. Histology cannot provide a clear understanding on how glands in adenocarcinomas connect to each other. A three-dimensional approach might thus be interesting. In one study we, for instance, found that ill-formed glands are actually thinner versions of well-delineated glands, forming a similar kind of anastomosing network.[86] Fused glands are also rather similar to grade 3 glands, but contain more intertwining connections. Little is known about the three-dimensional relation between various types of prostate cancer growth patterns. Since the disease is so heterogeneous and complex to understand, this might be a worthwhile avenue to explore.

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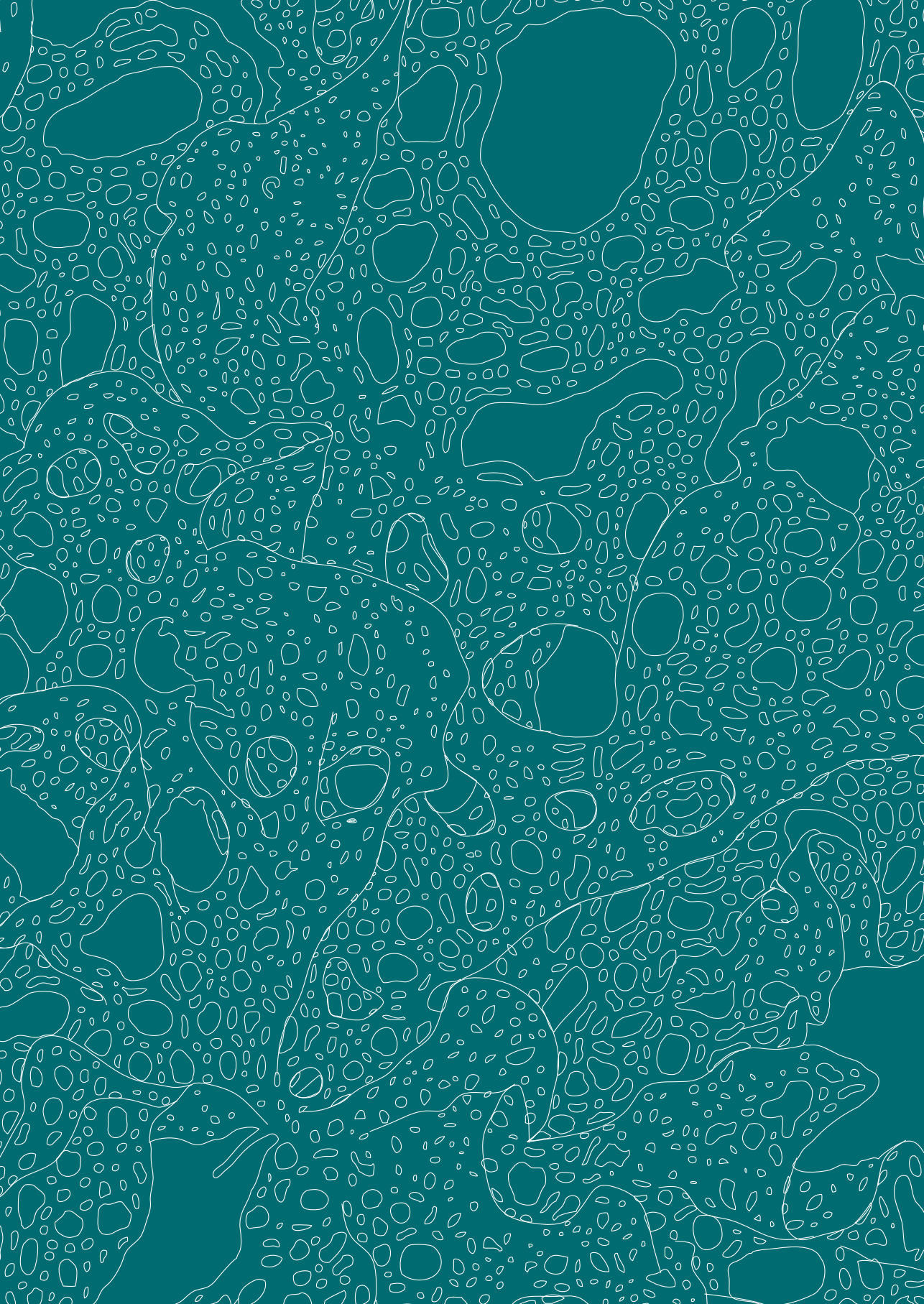
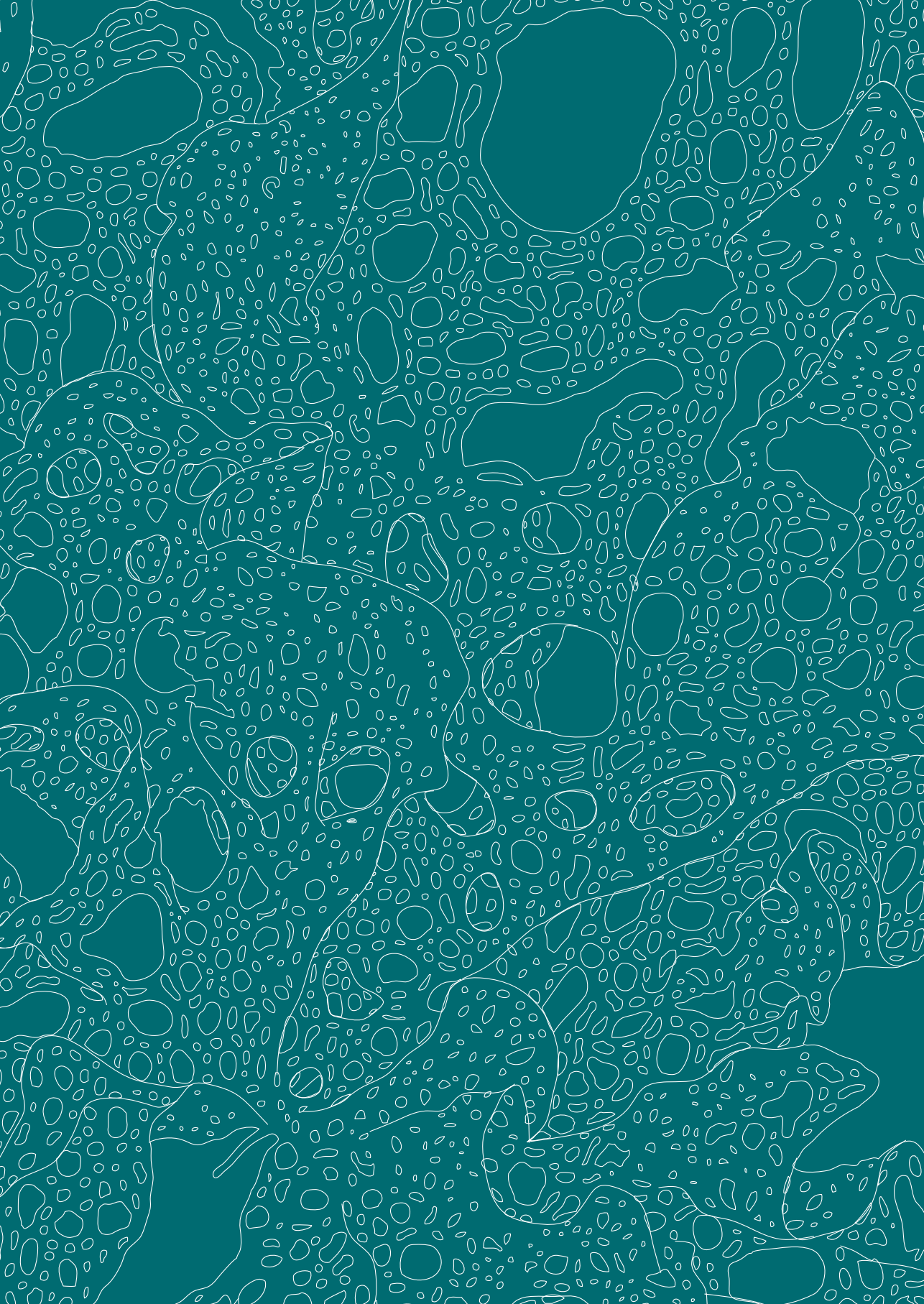


SUMMARY

The management of prostate cancer is clinically challenging, because of its variability in histomorphology, genetics and clinical outcome. Clinical-decision making currently depends upon serum prostate-specific antigen (PSA) level, clinical tumor stage, and a biopsy Gleason score given by the pathologist. The Gleason grading system is based on the architectural tumor growth patterns. In needle biopsies, the score is based on the sum of the most frequent and highest growth pattern, for instance 3+4=7. In radical prostatectomy specimens the score is based on the two most common patterns. Patients with the lowest Gleason scores (5 and 6) have an excellent outcome, while those the highest Gleason scores (9 and 10) have the worst. In general, patients with Gleason score 6 (3+3) on needle biopsy do not need immediate treatment and are often candidates for active surveillance. Patients with Gleason score 7 or higher generally undergo active treatment, i.e. surgery or radiotherapy. Although the Gleason score remains one the most important prognostic factors in prostate cancer, patient outcomes of those with Gleason score 7 or higher still vary considerably. Improving risk assessment of Gleason score 7 patients is of particular interest, as this score on biopsy represents an important clinical threshold for active treatment. In the past decade the Gleason grading system has, however, been revised twice leading to significant grade inflation. From a pathological perspective, the current Gleason score 7 (3+4 or 4+3) prostate cancer population represents a mixture of various histomorphological growth patterns that, as a whole, are regarded high grade. This thesis focuses on several pathological challenges of prostate cancer that have been insufficiently addressed: the prognostic value of individual high-grade histomorphological growth patterns, their inter-observer reproducibility and their association with genetic abnormalities.

Chapter 1 begins with an introduction on prostate cancer and provides a background of previous studies. It additionally describes the aims and outlines of this thesis. **Chapter 2** shows that patients with modified Gleason score ≤6 that had been treated by radical prostatectomy have an excellent clinical outcome and do not develop metastatic disease later in life or die from their disease. We also describe that a significant amount of patients with classic Gleason score ≤6 that had an adverse clinical outcome after surgery had presence of cribriform tumor glands at pathological revision of the specimen. In **chapter 3** we investigated whether presence of specific histomorphological tumor growth patterns in Gleason score 7 patients are associated with an adverse outcome. We found that patients with presence of cribriform tumor glands had a significantly worse outcome, while those with fused or ill-formed glands performed much better. **Chapter 4** elaborates on the prognostic

value of cribriform growth, together with its mimicker intraductal carcinoma, in pre-treatment diagnostic needle biopsies. Here, we show that presence of invasive cribriform growth and/or intraductal carcinoma (CR/IDC) is associated with a worse clinical outcome in various Gleason grading groups. This study also shows that survival rates of Gleason score 3+4=7 prostate cancer in the absence of CR/IDC growth (7-) are similar to that of Gleason score 6, suggesting that these patients could be candidates for active surveillance. **Chapter 5** explores the latter two patients populations more in depth by specifically studying their tumor characteristics and association with prognosis in different treatment groups using biochemical recurrence as outcome measure. This study shows that men with biopsy Gleason score 7- prostate cancer have similar survival rates to those with Gleason score 6 after radical prostatectomy or radiotherapy and additionally supports the suggestion that these patients may be candidates for active surveillance. In **chapter 6** we discuss another potentially valuable pathological prognostic factor in diagnostic needle biopsies, i.e. percentage of Gleason grade 4 patterns, and analyze its prognostic value together with other contemporary clinically relevant variables and CR/IDC growth. Here, we show that CR/IDC growth outperforms percentage Gleason grade 4 in predicting clinical outcome of men with Gleason score 3+4=7 prostate cancer. To further underline the potential of cribriform growth as a clinically applicable prognostic marker, **chapter 7** describes an inter-observer reproducibility study on Gleason grade 4 growth patterns, showing that cribriform and glomeruloid patterns were mostly reproducible among pathologists, while fused and ill-formed were not. In recent years, presence of cribriform growth, genomic instability and several distinct copy number alterations have all been separately linked to aggressive prostate cancer and adverse patient outcome. In **chapter 8** we close the circle and provide evidence that cribriform growth in prostate cancer is linked to genomic instability and copy number aberrations in distinct genomic regions that had been previously associated with an aggressive clinical course. Finally, in **chapter 9** we summarize our main findings and review their interpretation in the context of the current knowledge. We also provide a discussion on methodological considerations, clinical applications and recommendations for future research.

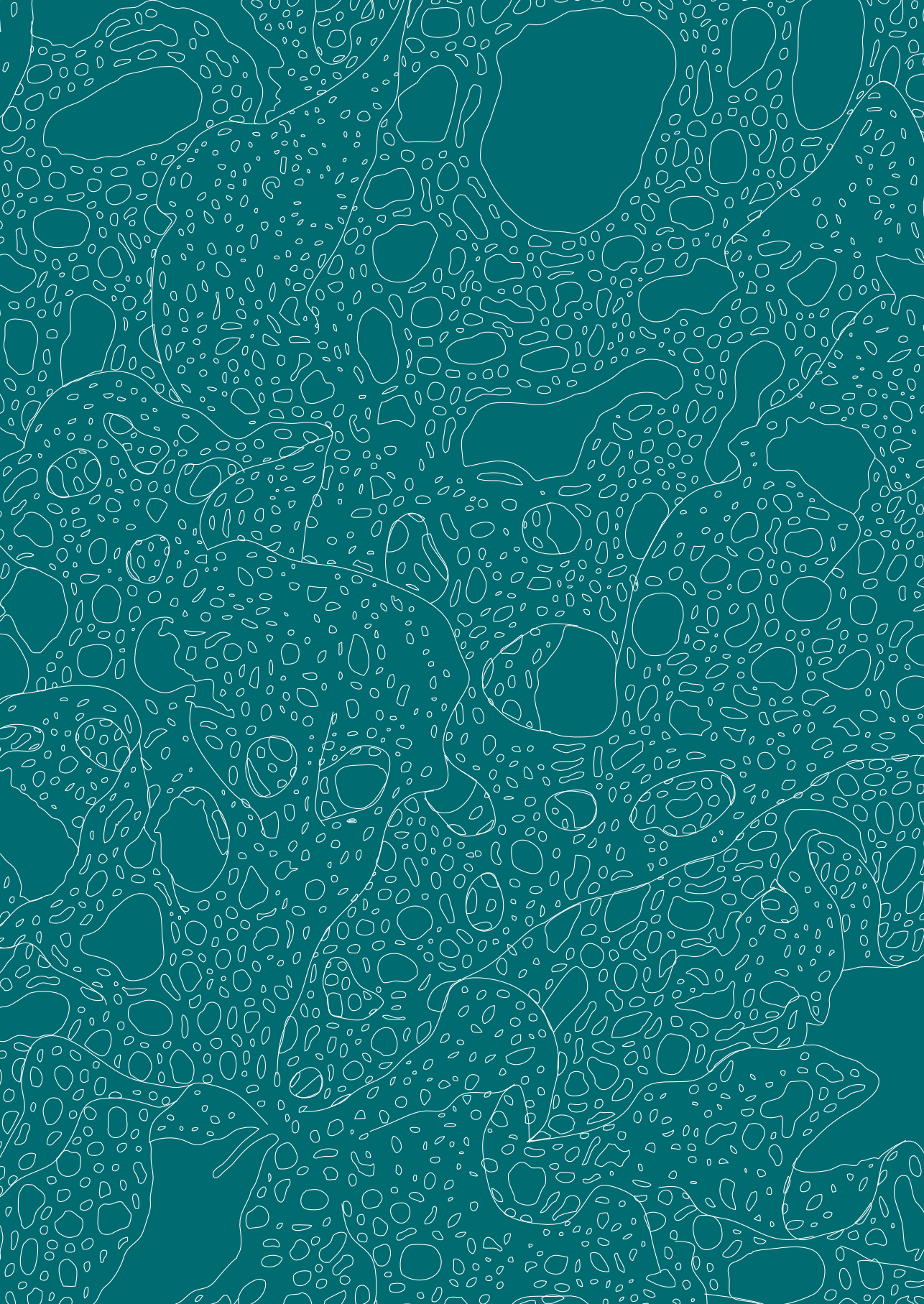


SAMENVATTING

De zorg omtrent prostaatkanker is een klinische uitdaging vanwege de variabiliteit in histomorfologie, genetica en patiëntenuitkomst. Tegenwoordig hangt de klinische beslisvorming af van het serum prostaat-specifieke antigen (PSA), klinische tumorstadium en de Gleason score op het biopt, gegeven door de patholoog. De Gleason gradering is een system gebaseerd op architecturale groeipatronen van de tumor. In diagnostische naaldbiopten is de score gebaseerd op de som van het meest voorkomende en hoogste groeipatroon, bijv. 3+4=7. In radicale prostatectomie preparaten is de score gebaseerd op de som van de twee meest voorkomende groeipatronen. Patiënten met de laagste Gleason scores (5 en 6) hebben een uitstekende uitkomst, terwijl degenen met de hoogste Gleason scores (9 en 10) de meest ongunstige hebben. Over het algemeen worden patiënten met een Gleason score 6 (3+3) op het biopt niet direct actief behandeld, i.e. chirurgie of radiotherapie, maar zijn zij vaak kandidaten voor actieve surveillance. Patiënten met Gleason score 7 of hoger worden doorgaans wel actief behandeld. Hoewel de Gleason score een van de meest belangrijke prognostische factoren is in prostaatkanker, varieert de prognose van patiënten met Gleason score 7 of hoger nog sterk. Het verbeteren van de risicoschatting in Gleason score 7 patiënten is met name relevant, omdat deze score een klinisch afkappunt is voor actieve behandeling. In de laatste twee decennia is het Gleason graderingssysteem echter tweemaal gereviseerd wat uiteindelijk heeft geleid tot een significante graadinflatie. Vanuit een pathologisch perspectief bestaat de huidige Gleason score 7 (3+4 of 4+3) prostaatkankerpopulatie uit een mix van verscheidene architecturale groeipatronen die, in hun geheel, worden beschouwd als hooggradig. Dit proefschrift benadrukt verschillende pathologische uitdagingen omtrent prostaatkanker die tot op heden onvoldoende zijn behandeld: de prognostische waarde van individuele hooggradige groeipatronen, hun interobserver variabiliteit en hun associatie met genetische afwijkingen.

Hoofdstuk 1 begint met een introductie over prostaatkanker en geeft achtergrondinformatie van voorgaande studies. De doelstellingen en opbouw van dit proefschrift worden hier eveneens beschreven. **Hoofdstuk 2** beschrijft dat patiënten met een gemodificeerde Gleason score ≤6 die behandeld zijn middels radicale prostatectomie een uitstekende prognose hebben en geen uitzaaiingen ontwikkelen of sterven aan de gevolgen van prostaatkanker. We laten ook zien dat een significante hoeveelheid van de patiënten met een klassieke Gleason score ≤6 met een ongunstige prognose na de operatie aanwezigheid van het cribriforme groeipatroon had bij pathologische revisie van het preparaat. In **hoofdstuk 3** onderzochten wij of de aanwezigheid van specifieke tumorgroeipatronen in

Gleason score 7 patiënten zijn geassocieerd met een ongunstige prognose. Wij vonden dat patiënten met aanwezigheid van het cribriforme groeipatroon een significant slechtere prognose hadden, terwijl degenen met gefuseerde of grillige groeipatronen een zeer goede uitkomst hadden. **Hoofdstuk 4** werkt de prognostische waarde van cribriforme groei samen met een patroon dat deze nabootst - intraductaal carcinoom - verder uit in diagnostische naaldbiopten. In deze studie laten we zien dat de aanwezigheid van cribriforme groei en/ of intraductaal carcinoom (CR/IDC) sterk is geassocieerd met een ongunstige prognose in verschillende Gleason score groepen. Wij laten eveneens ziens dat de overlevingskansen van Gleason score 3+4=7 patiënten zonder CR/IDC (7^-) gelijk zijn aan die van Gleason score 6. Wij suggereren vervolgens dat deze patiënten kandidaten voor active surveillance zouden kunnen zijn. **Hoofdstuk 5** gaat dieper in op de twee laatstgenoemde patiëntenpopulaties door specifiek te kijken naar tumorkarakteristieken en associaties met prognose in verschillende behandelingsgroepen met biochemisch recidief als uitkomstmaat. Deze studie laat zien dat mannen met Gleason score 6 en 7^- prostaatkanker gelijke overlevingskansen hebben na radicale prostatectomie en radiotherapie en aanvullend ondersteunt dat Gleason score 7- patiënten kandidaten zouden kunnen zijn voor actieve surveillance. In **hoofdstuk 6** bespreken wij een andere potentieel waardevolle pathologische variabele in diagnostische naaldbiopten, i.e. percentage Gleason graad 4 patronen, en analyseren diens prognostische waarde tezamen met andere hedendaagse klinisch-relevante variabelen en CR/IDC groei. In deze studie laten wij zien dat CR/IDC groei beter presteert dan percentage Gleason graad 4 in het voorspellen van patiëntenuitkomst in Gleason score 7 patiënten. Om de potentie van cribriforme groei als een klinisch-relevante prognostische marker verder te ondersteunen, geeft **hoofdstuk 7** een studie weer naar de interobserver reproduceerbaarheid van Gleason graad 4 patronen, waarbij wordt aangetoond dat cribriforme en glomeruloïde patronen reproduceerbaar zijn tussen pathologen, terwijl grillige en gefuseerde patronen dat niet zijn. In recente jaren zijn de aanwezigheid van cribriforme groei, genomische instabiliteit en verschillende variaties in genkopieën los van elkaar geassocieerd met een agressief klinisch beloop. In **hoofdstuk 8** maken we de cirkel rond en laten we zien dat cribriforme groei in prostaatkanker geassocieerd is met genomische instabiliteit en variaties in genkopieën in specifieke genomische regio's welke in voorgaande studies gelinkt werden aan ongunstige prognose. Tot slot worden in **hoofdstuk 9** de algemene resultaten van dit proefschrift besproken en worden de bevindingen in een bredere context uitgelegd. Ook bespreken we methodologische overwegingen van onze studies, klinische toepassingen en ideeën voor toekomstig onderzoek.



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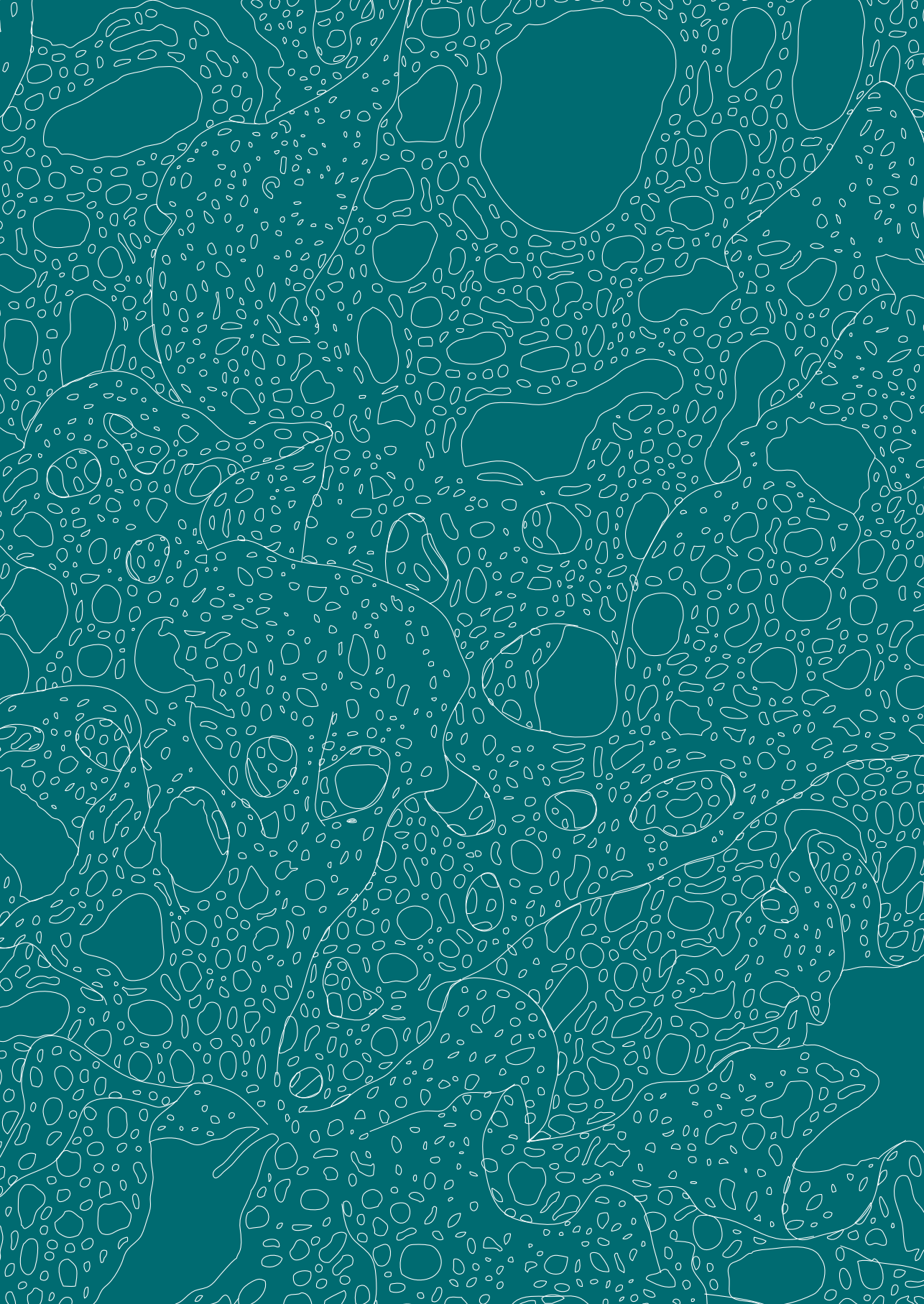
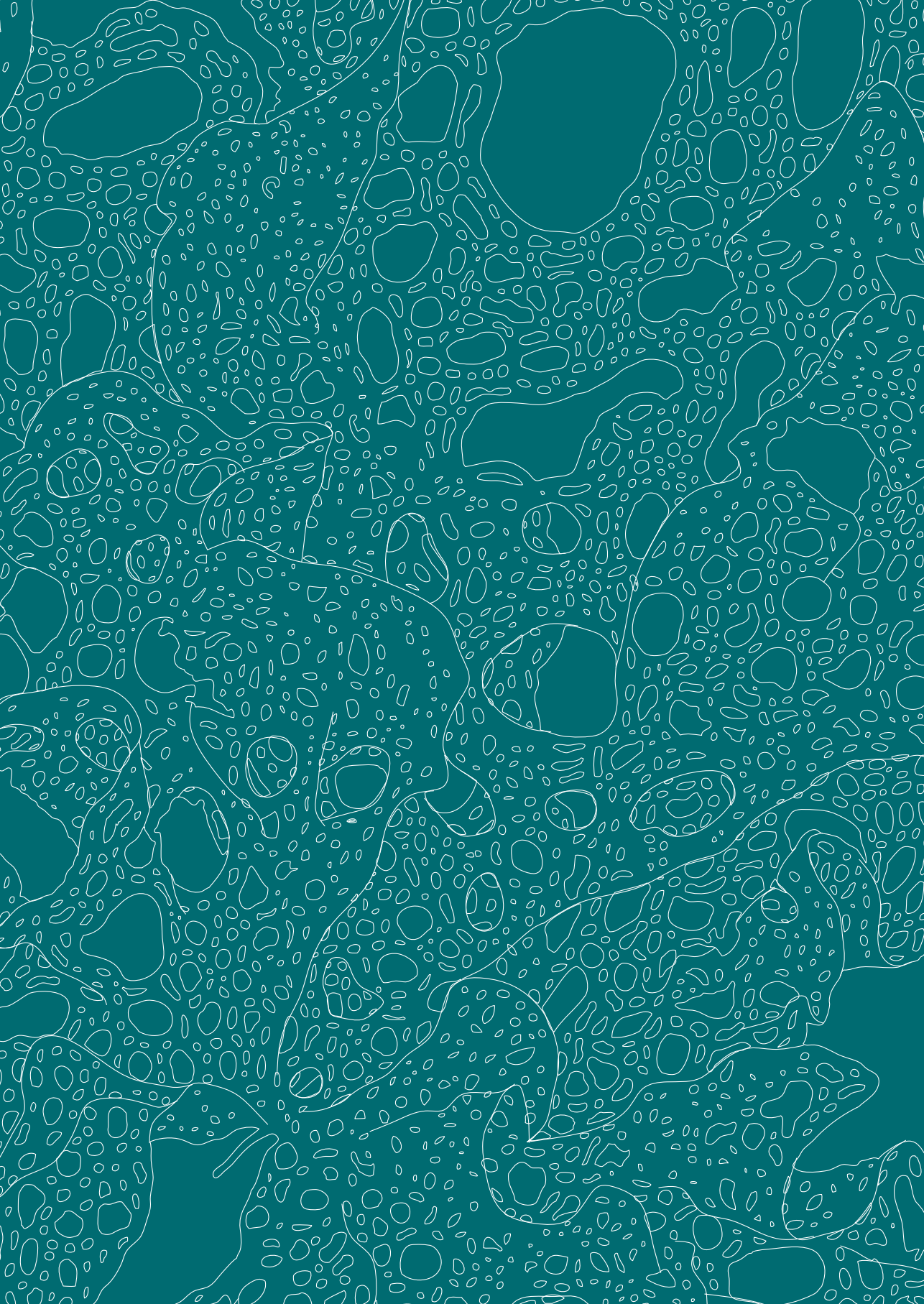


CURRICULUM VITAE

Charlotte Kweldam werd geboren op 10 juli 1988 te Vlaardingen. In 2006 behaalde zij haar eindexamen aan het Stedelijk Gymnasium te Schiedam. In hetzelfde jaar begon zij met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. In 2012 behaalde zij haar arts-examen na een keuze co-schap op de afdeling Radiologie van het Johns Hopkins Hospital te Baltimore in de Verenigde Staten en oudste co-schap op de afdeling Pathologie in het Erasmus MC te Rotterdam. In februari 2013 startte Charlotte met de opleiding tot patholoog in het Erasmus MC te Rotterdam. Na acht maanden onderbrak zij tijdelijk de opleiding vanwege promotieonderzoek op de afdeling Pathologie van het Erasmus MC onder supervisie van dr. G.J.L.H. van Leenders. In april 2016 hervatte zij de opleiding en verwacht zij begin 2020 haar specialisatie tot patholoog af te ronden.



PHD PORTFOLIO

Name PhD student:	Charlotte F. Kweldam
Department:	Pathology
Research school:	Molecular Medicine
PhD period:	2013-2016
Promotor:	prof. dr. F.J. van Kemenade
Copromotor:	dr. G.J.L.H. van Leenders

	Year	Workload (ECTS)
General courses		
Biomedical Research Techniques	2013	1.5
Course on Photoshop and Illustrator CS5	2013	0.3
Basic Human Genetics Course	2013	0.5
Basic Introduction Course on SPSS	2013	1
Survival Analysis Course	2013	0.5
Biomedical English Writing course	2014	2
Research Integrity	2014	0.3
Course on InDesign CS6	2014	0.3
Course on R	2015	1.4
English Biomedical Writing and Communication	2015	3
Specific courses		
NVVP long- en mediastinale pathologie	2013	0.3
LPAV cursus	2013-2016	1.5
A broad spectrum of NGS applications in Molecular Medicine	2014	2
Whole Genome Sequencing Course	2014	0.3
EACR-OECI joint training course: molecular mathology approach to cancer	2014-2016	1.5
Analysis of microarray and RNA Seq expression data using R/BioC	2014	2
Course on Molecular Diagnostics IX	2014	1
NGS in DNA Diagnostics Course	2014	1.5
SNP Course XI: SNPs and Human Diseases	2014	2
Oral presentations		
Pathology Laboratory Meetings	2013-2016	1.5
Urology Research Meetings	2013-2016	3
JNI Meetings	2013-2016	1.5
NVU voorjaarsvergadering/najaarsvergadering (Vlietstraprijs)	2014-2016	1

ECP Annual Congress	2015	1
AAV Wetenschapsmiddag (Award best presentation)	2015	0.3
Daniel den Hoed day	2016	0.3
ECP Annual Congress (Award best presentation)	2016	0.8

Poster presentations

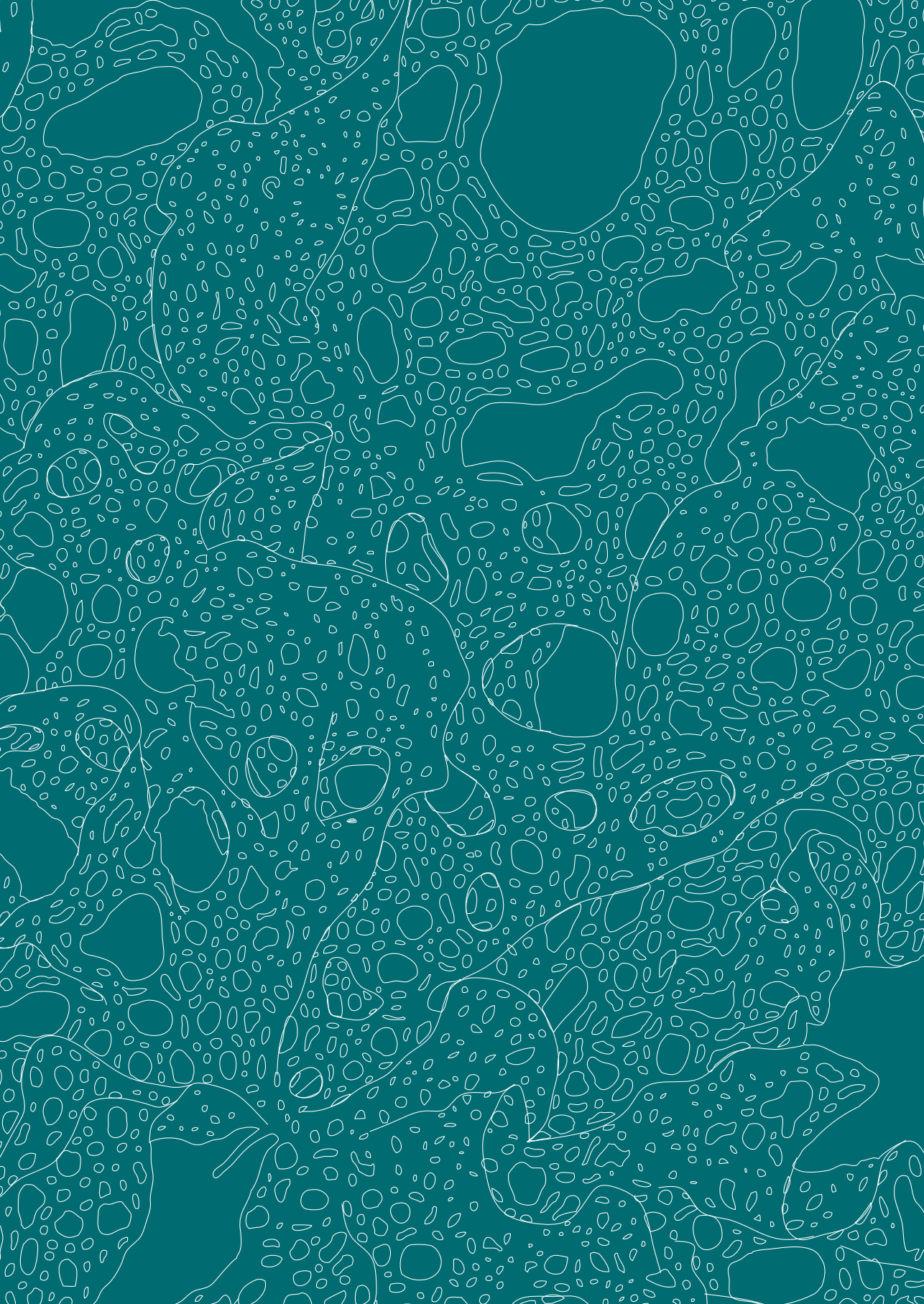
Pathologendagen	2014	1.5
ESUR Annual Meeting	2014	1.5
EAU Annual Congress	2016	1.5
USCAP Annual Meeting	2016	1.5

Conferences

Pathologendagen	2014	1.5
ESUR Annual Meeting	2014	1.5
Daniel100 Symposium	2014	0.5
Annual CGC.nl meeting	2014-2016	1.5
Bijeenkomst Moleculaire Diagnostiek in de Pathologie	2014-2016	0.9
NVU voorjaarsvergadering/najaarsvergadering	2014-2016	2
ECP Annual Congress	2015-2016	2
Prostaatdag	2015	0.3
Daniel den Hoed day	2016	0.5
USCAP Annual Meeting (F. Stephen Vogel Award)	2016	0.5
EAU Annual Congress (faculty member)	2016	2

Lecturing

VO Microsopische bouw mannelijke genitaliën	2013	0.5
VO Pathologie kleincellig & niet-kleincellig longcarcinoom	2014	0.5
VO Microscopische anatomie: pathologie van de luchtwegen	2014-2016	1.5
VO Microscopische anatomie: pathologie bot	2016	0.5



DANKWOORD

Op deze laatste bladzijde rest mij iedereen die aan de totstandkoming van dit proefschrift heeft bijgedragen te bedanken voor hun steun en medewerking. Alleen in Rotterdam al zijn veel afdelingen bij mijn onderzoek betrokken geweest, waaronder de collega's van de Pathologie, Urologie, Bioinformatica, Radiotherapie, Maatschappelijke Gezondheidszorg en het Cancer Computational Biology Center. Ook de mensen van de internationale samenwerking met Toronto in Canada van onder andere Laboratory Medicine Program en Informatics & Biocomputing Program ben ik veel dank verschuldigd. Leden van de kleine commissie wil ik bedanken voor het beoordelen van mijn proefschrift en leden van de grote commissie voor hun deelname aan de oppositie. Ook wil alle coauteurs bedanken. Om niet te vergeten mijn lieve vrienden, vriendinnen (vooral Lucia en Jessica, vriendinnen vanaf het 1e studiejaar), ouders, broers, schoonfamilie en natuurlijk Roderick. Een aantal personen wil ik in het bijzonder bedanken. Mijn copromotor, dr. G.J.L.H. van Leenders, beste Arno, dank voor je goede begeleiding, adviezen en vrijheid die je me hebt gegeven. Ik blik met veel plezier terug op een mooie samenwerking. Mijn promotor, prof. dr. F.J. van Kemenade, beste Folkert, dank voor uw betrokkenheid, wijze raad en steun bij het afronden van het proefschrift. Prof. dr. Th.H. van der Kwast, beste Theo, wat bijzonder dat je ondanks de grote afstand zo nauw betrokken was bij mijn onderzoek. Ik wil je bedanken voor je enthousiasme, deskundigheid en scherpe visie. Dr. I.P.E.D. Kümmerlin, lieve Intan, het werk in dit proefschrift is voor een groot deel aan jou te danken. Samen hebben we vele uren aan de microscoop doorgebracht en eindeloos veel glaasjes beoordeeld. Een solide basis voor ons onderzoek, maar ook voor onze vriendschap. Dr. R. Böttcher, beste René, wat ooit begon als een idee voor een korte correspondentie in een gerenommeerd tijdschrift resulteerde in een jarenlange odyssee. Ik wil je bedanken voor je enorme inspanning en volhardendheid bij dit project. Mijn onderzoeksgroepgenoten, Esther en Kimberley, wil ik bedanken voor hun betrokkenheid, hulp en sympathie. Met een glimlach denk ik terug aan die wonderlijke momenten op kamertje 301. Als laatste wil ik mijn paranimfen bedanken. Lieve Kim, mijn maatje, we hebben elkaar leren kennen tijdens het onderzoek en in de afgelopen jaren een dierbare vriendschap opgebouwd. Ik heb bewondering voor je moed, daadkracht en gevoel voor humor. Het promotieonderzoek met jou was een spannende en dolkomische rit van begin (Schotland) tot eind (Spanje). Lieve Stéphanie, mijn oudste vriendin, bedankt dat je mijn paranimf wil zijn. Dit heeft voor ons beide een speciale betekenis. Bij het behalen van ons eerste diploma stonden wij in badpak naast elkaar. Meer dan twintig jaar later sta je opnieuw aan mijn zijde. Zullen we voor deze gelegenheid onze zwemspullen maar thuislaten?