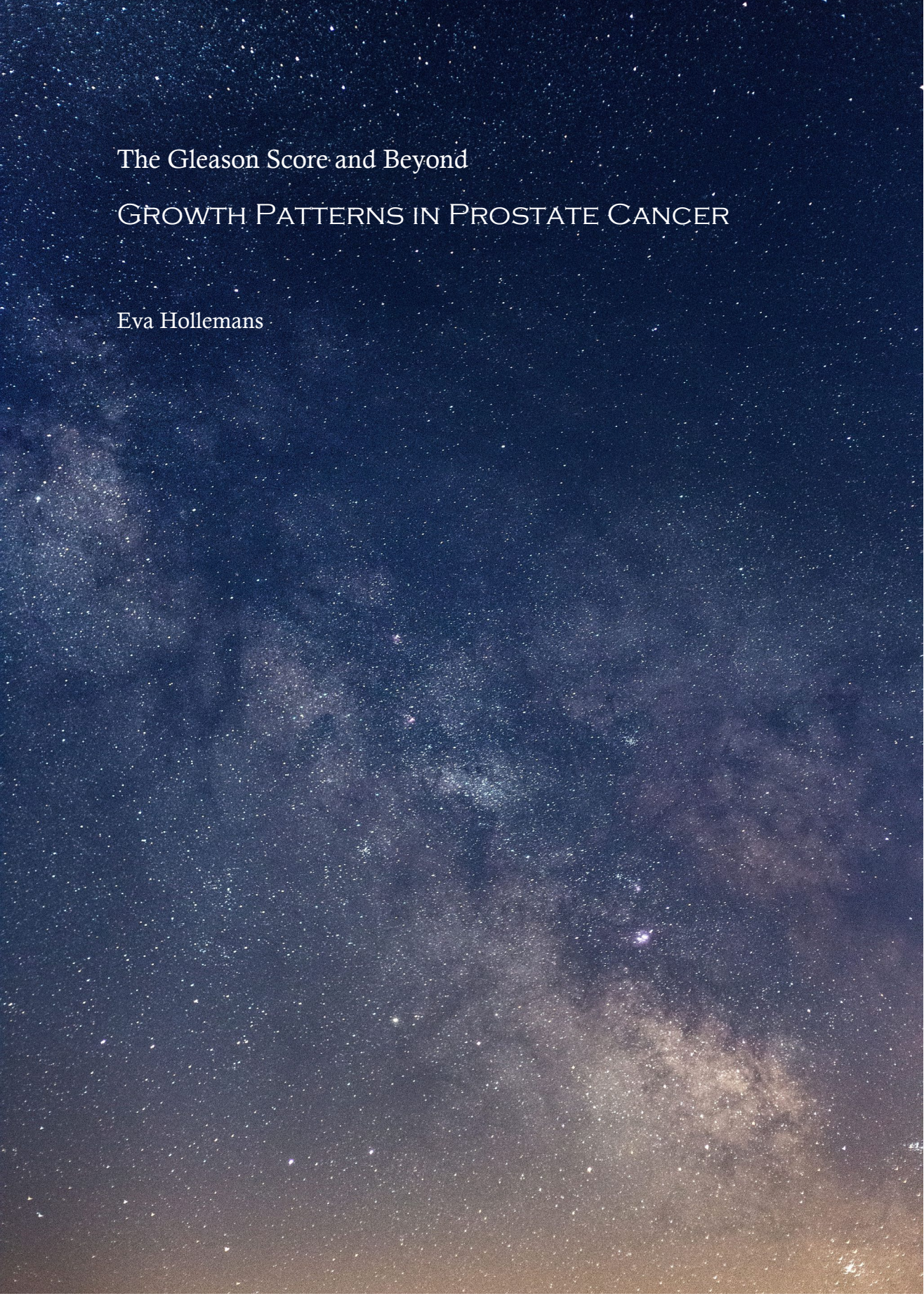

The Gleason Score and Beyond

Growth Patterns in Prostate Cancer

Eva Hollemans

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E. Hollemans

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Chapter image 'Jupiter Blues' by Gerald Eichstädt and Sean Doran, based on photographs made by spacecraft Juno, provided courtesy of NASA/JPL-Calltech/SwRI/MSSS.

The research described in this thesis was conducted at the Department of Pathology, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands.

Printing of this thesis was kindly supported by the Jaap Schouten Foundation, Brompton Bicycle and Erasmus Medical Center.

Jaap Schouten Foundation

The Gleason Score and Beyond
Growth Patterns in Prostate Cancer

De Gleason score en daar voorbij
Groeipatronen in prostaatkanker

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag van de rector magnificus

Prof. Dr. F.A. van der Duijn Schouten

En volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
11 mei 2021
om 15.30 uur
door
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Erasmus University Rotterdam

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Contents

Chapter I

General introduction and aims of the thesis.

The prostate gland

The prostate gland is an organ of the male reproductive system. It is located just below the bladder, around the urethra. In adolescents, the prostate is the size of a walnut and grows larger with age. The prostate fluid consists of enzymes that together with fluid from the seminal vesicles on the upper side of the prostate, forms an alkaline liquid to aid motility of spermatozoa.

Histologically, the prostate consists of glands covered by two layers of epithelium, lying within fibromuscular stroma. The secretory luminal cells are inner, cuboidal to columnar cells with small round nuclei an no or inconspicuous nucleoli. Among the secretory products is the prostate specific antigen (PSA). The basal cells are the outer, flattened cells surrounded by a basement membrane. The prostatic and ejaculatory ducts flow into the urethra, running through the centre of the prostate. Neurovascular bundles run from apex to base at the lateral edges of the prostate.[1]

Brief history of prostate cancer

Cancer represents a paradox in our modern age. Although there is remarkable faith in our biomedical capability, we fail to comprehend the nature of cancer. The rise of morbid anatomy in the 16th century and postmortem pathology in the 18th century was the first leap forward in understanding the aspects of death and disease.

Andreas Vesalius was a physician from the Habsburg Netherlands, who was determined to investigate the human body based on careful observation, since medical knowledge at that time was not sufficiently based on human dissection. He wanted to open and read the body for himself. His tremendous objective was finalized in 1543 with the publication of *De Humani Corporis Fabricia.* The books became well-known. Herein the prostate gland is illustrated for the first time. Vesalius had a crucial influence on the interest in postmortem anatomy. During the following centuries many tried to correlate clinical manifestations and postmortem

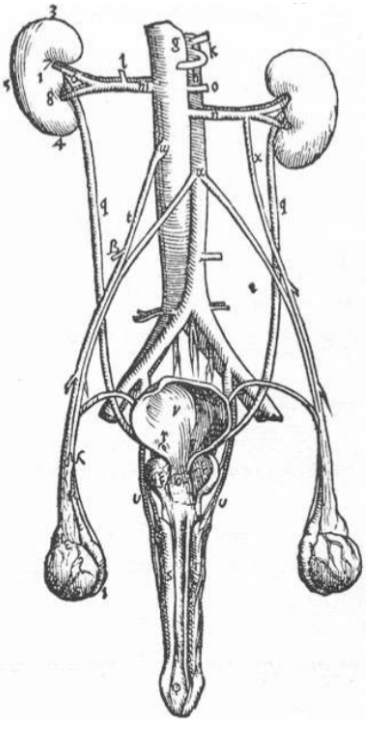

Figure 1.1. Male genitalia, anterior view. Vesalius, A. D*e Humani Corporis Fabricia*, fig. XXIII, 1543.

observations. However, descriptions of the prostate were not sufficient enough to determine the nature of disease in men with urinary symptoms.

Change came in the 19th century. When in 1853 dr. Adams wrote a letter about how his colleagues and he had come across a "very rare, scirrous disease" of the prostate, it must have been implausible to him that over 150 years later, it would be among the most common male malignancies.[2] Earlier possible cases of prostate cancer were described, however unfortunately, the true origin of anomalies could not be determined by gross inspection alone.[3] Examination of the body in dr. Adam's case showed a scirrous tumour, meaning of firm and fibrous consistency, in the left lobe of the prostate. Dr. Adams was able to consult a microscopist, who had declared it to be 'true scirrous in every particular'. When he showed his colleagues the growth was also present in iliac glands, all uncertainty regarding its nature was brought to an end.

Clinical approach

In the Netherlands, the incidence of prostate cancer is rising. Prostate cancer affected 12.646 men in 2018.[4] The lifetime chance of prostate cancer diagnosis is 11%.[5] Usually prostate cancer is asymptomatic, rarely patients present with urinary symptoms or hematuria. The cause of these symptoms, however, often lies with benign prostatic hyperplasia (BPH). BPH is a common disease among elderly men, affecting 50% of men at age 50 and 80% of men at age 80.[6] Elevated PSA levels and abnormal findings on digital rectal examination raise clinical suspicion for prostate cancer. Currently, prostate cancer is diagnosed using biopsies taken with multiresonance imaging (MRI) and/or ultrasound. When the pathologist confirms the diagnosis of cancer on biopsies, patients become eligible for either surveillance or active treatment.

Although prostate cancer has a high prevalence and it is the secondary cause of death among males, most men will not die from their disease. Low risk prostate cancer is a slow growing malignancy, unlikely to decrease life expectancy. Therefore, active surveillance has become a widely acceptable alternative for surgery in patients with low risk prostate cancer. With the use of risk calculation models, patients who will not benefit from therapy are identified and monitored instead.[7] A bi-annual urologists' appointment with measurement of PSA levels and a digital rectal examination are used to monitor and detect disease progression. Also, repeat prostate biopsies are taken when PSA levels rise.

Surgery is a common treatment for intermediate to high risk prostate cancer. Laparoscopic robot-assisted radical prostatectomy is a curative strategy, however considerable potential side effects are urinary incontinence and erectile dysfunction. Other treatment strategies include radiation or hormone therapy. Radiation therapy can be used as initial treatment for low grade tumours as well as high grade tumours, and for tumours growing outside the prostate. When

prostate cancer surgery did not succeed in complete removal of the malignancy, or in case of disease progression, men are treated with radiation therapy. Radiation affects the surrounding tissues as well and may cause symptoms of bladder and bowel injury, sexual dysfunction, skin irritation and fatigue. Hormone therapy interferes with the need of prostate cancer cells for male hormones. Androgens are growth stimuli for prostate cancer cells. Deprivation of these hormones causes reduced growth and even shrinkage of the malignancy. Both radiation and hormone therapy are often used without curative intent, when the prostate cancer is too widespread, to alleviate symptoms and prolong life expectancy.

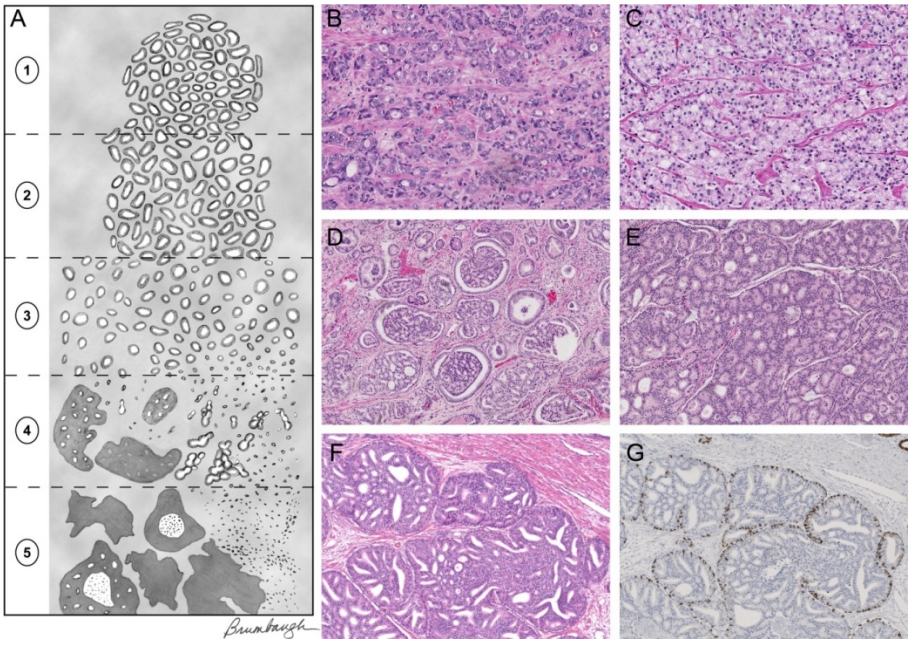

Figure 1.2. A. Modified Gleason grading system based on the ISUP 2014 consensus meeting. B-E. Gleason grade 4 growth patterns with ill-formed (B), fused (C), glomeruloid (D) and cribriform glands (E). F-G. Intraductal carcinoma can mimic invasive cribriform carcinoma on HE staining (F), however, basal cell immunohistochemistry using 34BE12 shows presence of basal cells (G).

Pathology

For decades, the Gleason grading system has been the fundamental way for pathologists to classify prostate cancer. In 1966, dr. Donald Gleason developed the histological classification based solely on architectural growth patterns of prostate cancer, rather than by cytological nuclear atypia, to bring order into the morphological heterogeneity.[8] Within the same tumour, different architectural growth patterns can be identified. Dr. Gleason distinguished five elementary patterns and suggested each tumour would be assigned two patterns: the primary, most common architectural pattern, followed by the secondary pattern. After validation, the Gleason grading system was incorporated in pathology departments worldwide and is one of the most important predictive parameters in prostate cancer outcome.[9, 10] The International Society for Urological Pathology (ISUP) modified the grading system during consensus meetings in 2005 and 2014, leading to the Gleason score as known today.[11, 12]

- Gleason grade pattern 1 and 2 were initially described as well differentiated glands, with little variation in size, forming a circumscribed tumour mass. Some cases later appeared to be mimickers of cancer. This, together with histomorphological similarity to pattern 3 and the inability to distinguish these patterns on biopsies, made the patterns obsolete. Therefore, nowadays the lowest possible score of prostate cancer according to the Gleason grading system is 3 + 3 = 6.
- Gleason grade pattern 3 consist of well delineated invading glands, with marked variation in size and shape and lined with one layer of epithelial cells. The epithelial cells show slightly pale or basophilic cytoplasm and mild to moderate atypia with enlarged nuclei and visible nucleoli.
- Gleason grade pattern 4 comprises four major growth patterns. Ill-defined glands are irregular with poorly formed lumina. Fused glands form interconnecting structures with increased complexity. Cribriform growth shows a field of glands with punched out lumina without intervening stroma. Glomeruloid glands resemble the glomerulus of the kidney. They are dilated glands wherein a proliferation of tumour cells is present, attached to one side of the gland wall. The proliferation might have a cribriform aspect.
- Gleason grade pattern 5 is devoid of glandular differentiation and is composed of single cells, cords or sheets of cells. Pattern 5 may also show solid or cribriform fields with comedonecrosis.

In 2016, the World Health Organization (WHO) and ISUP proposed a grouping system, based on the modified Gleason grading system, in order to distinguish clinically significant patient groups.[12]

The Grade Groups comprise 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) and Grade Group 5 (Gleason score 9 and 10).

Cribriform prostate cancer

Last decade, cribriform growth in prostate cancer has been recognized as highly significant subtype of Gleason grade pattern 4. In 2011, Iczkowski et al. were the first to relate cribriform growth to adverse outcome.[13] They reported worse biochemical recurrence-free survival in patients with cribriform prostate cancer compared to patients without the pattern. To date, cribriform growth has been linked to advanced stage, worse biochemical recurrence-free survival, metastasis and disease-specific death in biopsies as well as in radical prostatectomy specimens.[14-17] Cribriform carcinoma is associated with increased genomic instability and harbours distinct genomic alterations.[18] Remarkably, cribriform growth pattern used to belong in the Gleason grade 3 group, but was reassigned to grade 4 in 2005. Hitherto, the clinical significance of cribriform growth has been acknowledged. However, there is a need to elucidate heterogeneity among cribriform growth patterns and their individual prognostic value.

Although cribriform growth is a high risk pattern compared to ill-defined and fused growth patterns, little is known about the clinical significance of glomeruloid growth. Few studies reported on the glomeruloid pattern and they show contradictory results.[16, 19] Lotan et al. reported an association between glomerulations and concurrent high grade carcinoma and cribriform growth on prostate biopsies.[19] Others could not find an association with glomeruloid growth and worse outcome.[20, 21] The study of Kweldam et al. even showed a trend towards favourable outcome in Gleason score 7 prostate cancer on radical prostatectomy.[16]

The clinical significance of intraductal carcinoma of the prostate (IDC-P) has been acknowledged as well, although this lesion is not incorporated in the 2014 Gleason grading system. Intraductal carcinoma is defined as an expansile proliferation of atypical secretory epithelial cells within pre-existent prostatic ducts.[22, 23] Intraductal carcinoma often shows cribriform architecture, but may also have solid or papillary appearance. Invasive cribriform carcinoma and cribriform intraductal carcinoma of the prostate can be distinguished by the presence of basal cells surrounding intraductal carcinoma, since they belong in the normal gland epithelium. Presence of intraductal carcinoma has been associated with advanced tumour stage, concurrent high grade invasive carcinoma and worse outcome, including biochemical recurrence and metastasis.[24-28] The origin of intraductal carcinoma is yet unclear, as proposed concepts include retrograde glandular colonization from a common denominator with invasive cribriform growth as well as a premalignant precursor lesion [29, 30].

Aims of the thesis

The general scope of this thesis is to study histomorphological growth patterns in prostate cancer and to identify favourable parameters for intermediate and high grade prostate cancer. In more detail, the aims of this thesis are

- To study variants of cribriform growth, especially small and large invasive cribriform prostate cancer and intraductal cribriform prostate cancer in radical prostatectomy specimens. (Chapter 2)

- To investigate concordance of cribriform growth on biopsies and radical prostatectomy specimens and to identify predictive parameters for presence of cribriform growth. (Chapter 3)

- To study the prognostic value of cribriform-negative prostate cancer in intermediate risk cancer in radical prostatectomy specimens. (Chapter 4)

- To elucidate the morphology and effect on clinicopathological outcome of glomeruloid Gleason pattern 4. (Chapter 5)

- To stratify high risk prostate cancer according to presence of cribriform growth in radical prostatectomy specimens and investigate its impact on clinical outcome. (Chapter 6)

Chapter II

Large cribriform growth pattern identifies Grade Group 2 prostate cancer at high risk for recurrence and metastasis.

Hollemans E, Verhoef EI, Bangma CH, Rietbergen J, Helleman J, Roobol MJ, van Leenders GJHL.

Mod Path. 2019 Jan; 32: 139-146.

Abstract

Invasive cribriform and intraductal carcinoma are associated with adverse clinical outcome in patients with Gleason score 7 prostate cancer. It is yet unclear whether invasive cribriform and intraductal carcinoma of the prostate both have independent prognostic value, or whether field size of invasive cribriform carcinoma has impact on disease outcome. Our objective was to determine the prognostic impact of intraductal and invasive cribriform prostate cancer histological subtypes in radical prostatectomies.

We reviewed 420 prostatectomy specimens with Grade Group 2 prostate cancer, assessed the percentages of Gleason grade 4 and tertiary 5, and performed immunohistochemistry for basal cells to discriminate intraductal from invasive cribriform growth. Small and large invasive cribriform fields were distinguished based on a diameter of at least twice the size of adjacent pre-existent normal glands. Clinicopathological parameters and biochemical recurrence-free survival were used as endpoints.

Cribriform architecture was observed in 228 (54.3%) men, 103 (24.5%) of whom had intraductal, 194 (46.2%) small invasive and 34 (8.1%) large invasive cribriform growth. Large invasive cribriform architecture was associated with older age ($P<0.001$), higher percentage Gleason grade 4 ($P=0.001$), extraprostatic expansion ($P<0.001$) and more frequent lymph node metastases ($P=0.002$), when compared with small invasive cribriform and/or intraductal carcinoma. Univariate analysis identified PSA, pT-stage, surgical margin status, intraductal and invasive cribriform growth as significant predictors for biochemical recurrence-free survival. In multivariable Cox regression analysis, pT-stage (hazard ratio 1.64, 95%CI 1.02-2.63, $P=0.04$), positive surgical margins (hazard ratio 3.28, 95%CI 2.06-5.23, $P<0.001$) and large cribriform growth (hazard ratio 4.36, 95%CI 2.08-9.17, $P<0.001$) were independent predictors for biochemical recurrence-free survival, while intraductal carcinoma, small cribriform growth, and percentage of Gleason grade 4 were not.

In conclusion, large cribriform fields represent an aggressive sub-pattern of invasive cribriform prostate cancer and are an independent predictive factor for biochemical recurrence-free survival in Grade Group 2 prostate cancer patients.

Introduction

The Gleason score is one of the most important parameters for clinical decision-making in men with prostate cancer. The Gleason grading system is entirely based on tumour architectural growth patterns which are classified into five different grades. While men with biopsy Gleason score 6 are frequently eligible for active surveillance, treatment is warranted in patients with Gleason score 8-10. The optimal therapeutic strategy for individual patients with Gleason score 7 is not yet clear. While most patients with Gleason score 7 undergo radical prostatectomy or radiation therapy, active surveillance is increasingly being considered in this large group of men. Therefore, there is an urgent need for additional parameters to aid therapeutic decision-making in men with Gleason score 7 prostate cancer.

Gleason score 7 prostate cancer is composed of well-delineated Gleason grade 3 glands along with Gleason grade 4 structures. Gleason grade 4 prostate cancer is heterogeneous, comprising a range of growth patterns, categorised as poorly formed, fused, glomeruloid and cribriform.[12, 31] These individual growth patterns are generally not specified in pathology reports, however several studies have found that patients with invasive cribriform growth have a worse outcome than men without this pattern.[13-17, 32, 33] Among cribriform prostate cancers heterogeneity of architectural pattern is still present, with some areas being round and small, while others are large and confluent vastly exceeding pre-existent gland diameter.[11, 34]

Intraductal carcinoma of the prostate is characterised by either cribriform or solid malignant epithelial proliferation, or loose cribriform and micropapillary formations of severely atypical cells, in pre-existent large acini and prostatic ducts, with preservation of basal cells. [12] Although intraductal carcinoma is formally not included in the Gleason score, numerous studies have linked intraductal carcinoma to more aggressive disease.[25, 28, 34-37] The presence of intraductal carcinoma ought thus to be routinely noted in pathology reports.[12, 38]

Invasive cribriform Gleason grade 4 prostate cancer and intraductal carcinoma often coexist, and can be difficult to distinguish without the use of immunohistochemical staining of basal cells. At present, it is not clear whether invasive cribriform carcinoma and intraductal carcinoma both have independent prognostic value for prostate cancer, or whether invasive cribriform sub-patterns have additional prognostic value. [16, 39] The objective of this study is to determine the outcome of invasive cribriform sub-patterns and intraductal carcinoma in patients with Grade Group 2 after radical prostatectomy.

Materials and methods

Patient selection

In total 854 patients were identified who had undergone radical prostatectomy for prostate adenocarcinoma at the Erasmus MC, Rotterdam, The Netherlands between 2000 and 2017. Men who had undergone hormonal, radiation or viral therapy (n=19) prior to operation were excluded from this study.[40] After fixation in neutral-buffered formalin, the radical prostatectomy specimens were sectioned transversely and entirely embedded for diagnostic purposes. All slides and blocks were available for pathology review. The use of tissue samples for scientific purposes was approved by the institutional Medical Research Ethics Committee (MEC-2011-295, MEC-2011-296). Samples were used in accordance with the "Code for Proper Secondary Use of Human Tissue in The Netherlands" as developed by the Dutch Federation of Medical Scientific Societies (FMWV, version 2002, update 2011).

Pathologic evaluation

Two investigators blinded to clinical outcome (EH, GvL) reviewed all radical prostatectomy specimens (n=854). The following features were recorded: Gleason score according to the WHO/ISUP 2014 guidelines, pT-stage according to the AJCC TNM 8th edition, surgical margin status, presence of intraductal carcinoma, percentage Gleason grade 4, including specific growth patterns, and presence of tertiary Gleason grade 5.[12, 41] Based on this revision, Grade Group 2 specimens were identified. The following Gleason grade 4 growth patterns were recognised: poorly formed, fused, glomeruloid and cribriform glands.[12, 38] In addition, we distinguished small and large cribriform growth patterns. Small cribriform structures had a diameter less than twice the size of adjacent benign glands. Large cribriform pattern was defined as having a diameter of at least twice the size of adjacent pre-existent normal glands, and could either represent one large well defined cribriform field or a confluent cribriform area (Figure 2.1). Invasive cribriform Gleason grade 4 was morphologically distinguished from intraductal carcinoma based on the following features: invasive cribriform prostate cancer had irregular outline, showed anastomosing fields beyond pre-existent gland architecture or extension into periprostatic adipose tissue, ejaculatory ducts or seminal vesicles. Intraductal carcinoma was morphologically identified if cribriform structures were clearly continuous with pre-existent glands lined by normal basal epithelium, or containing corpora amylacea. Where invasive cribriform carcinoma and intraductal carcinoma could not be differentiated by morphological criteria alone, additional immunohistochemical staining for the presence of basal cells was performed; in total 261 slides

from 156 Grade Group 2 patients were stained. Gleason grade 5 was considered as a tertiary pattern if it occupied less than 5% of the total tumour area.[12, 31, 38]

Immunohistochemistry

Four micrometer thick tissue sections were cut from selected paraffin-embedded blocks and mounted on slides (Superfrost Microscopic Slides, ThermoFisher Scientific, Bleiswijk). Slides were deparaffinised and rehydrated with xylene and ethanol. Endogenous peroxidase was blocked using 0.3% H_2O_2 in PBS. Heat-induced antigen retrieval was accomplished by 15 min in Tris-EDTA buffer (pH 9; Klinipath, Duiven, The Netherlands). Mouse monoclonal high molecular weight cytokeratin (clone 34BE12; 1:200; DAKO; Heverlee, Belgium) diluted in normal antibody diluent (APG-500; ScyTek Laboratories, West Logan, USA) was incubated for 2h at room temperature. Antibody visualization was performed with Envision kit (DAKO) and slide counterstaining with hematoxylin. When basal cell staining was completely absent around a cribriform gland, it was categorised as invasive cribriform carcinoma; if sporadic, scattered or continuous basal cells were identified the structure was classified as intraductal carcinoma.

Clinical follow-up

Clinical follow-up after radical prostatectomy consisted of six-monthly, and later annual monitoring of serum PSA levels. Biochemical recurrence was defined as PSA level ≥ 0.2 ng/ml measured at two separate time points at least three months apart when PSA had been undetectable after operation, or as PSA increase of > 2.0 ng/ml if serum PSA had not declined to zero after operation. Post-operative lymph node and distant metastases were confirmed by biopsy or multidisciplinary consensus. Biochemical recurrence-free survival was defined as time in months from radical prostatectomy to biochemical recurrence.

Statistical analysis

Normally distributed, continuous variables were analysed using the independent sample Student's t-test, whereas variables without normal distribution were analysed using the Mann-Whitney U test. Pearson's chi squared (χ^2) test was used for categorical parameters. Percentage Gleason grade 4 was analysed both as continuous and dichotomous parameter (≥5% and <25% *versus* ≥25% and <50%). Missing PSA values (n=27) were imputated using the median PSA value. Biochemical recurrence-free survival was analysed using Cox proportional hazards regression and visualised by Kaplan-Meier curves, excluding patients with lymph node metastases at time of operation. Statistics were performed using SPSS version 24 (IBM, Chicago, IL, USA). Results were considered significant when the two-sided P-value was <0.05.

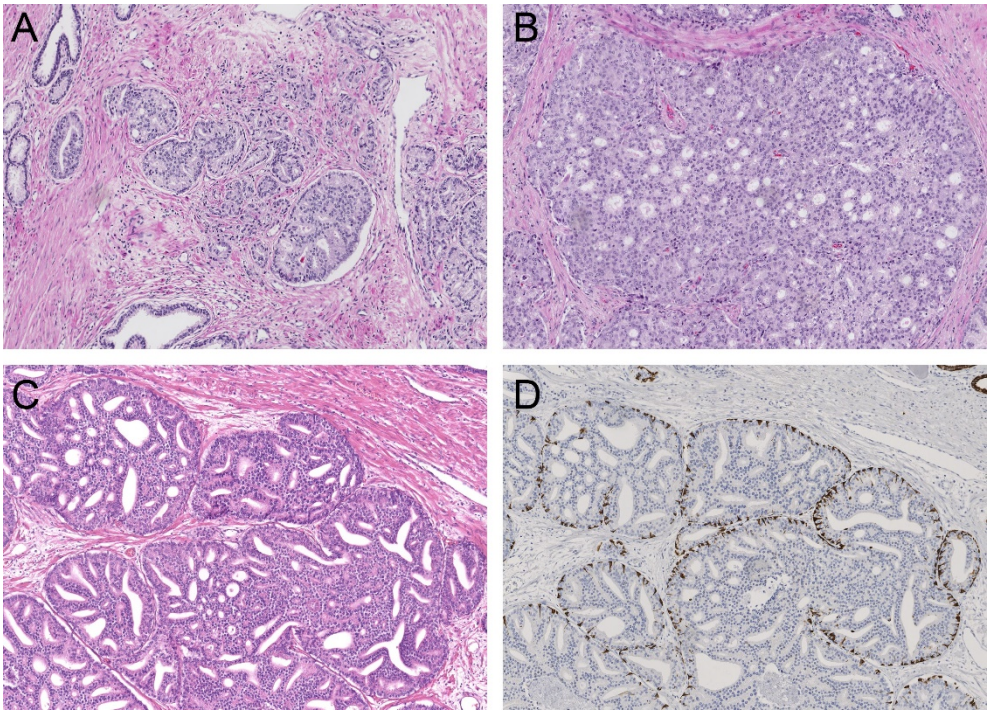

Figure 2.1. Gleason grade 4 cribriform growth patterns and intraductal carcinoma. A. Small invasive cribriform carcinoma, 10x. B. Large invasive cribriform carcinoma, 10x. C-D. Intraductal cribriform carcinoma, with presence of basal cells, 10x.

Results

Patient characteristics

Out of the 854 revised patients, 420 showed Grade Group 2 at radical prostatectomy and were included in this study. Median age at radical prostatectomy was 64.6 years (interquartile range 59.8-68.1) and median PSA level was 8.2 ng/ml (interquartile range 5.9-12.6). The tumour stage was distributed as follows: pT2 (n=234; 55.7%), pT3a (n=153; 36.4%) and pT3b (n=33; 7.9%). A positive surgical margin was present in 142 cases (33.8%). In total 241 men (57.4%) had undergone pelvic lymph node dissection at the time of radical prostatectomy; in 12 patients (2.9%) one or more lymph node metastases were present.

Poorly formed glands (n=325; 77.4%) were the most common Gleason grade 4 pattern followed by fused (n=290; 69.0%), cribriform (n=204; 48.6%) and glomeruloid (n=194; 46.2%) glands. Seventy-five patients (17.9%) had one Gleason grade 4 pattern, 152 (36.2%) two, 133 (31.6%) three and 60 (14.3%) four growth patterns. Tertiary grade 5 was present in 49 (11.6%) men.

In total, 228 (54.3%) patients showed either invasive or intraductal cribriform carcinoma. These patients had higher PSA levels (mean 12.2 ng/ml *versus* 9.4 ng/ml; P=0.006) than those without cribriform architecture. They also more frequently had extraprostatic extension (51.8% *versus* 35.4%; P<0.0001) and positive surgical margins (39.5% *versus* 27.1%; P=0.007). One hundred and fifty (65.8%) patients with cribriform architecture and 91 (47.4%) patients without cribriform architecture had undergone pelvic lymph node dissection at the time of radical prostatectomy. Twelve (8.0%) of the patients with cribriform architecture were found to have lymph node metastasis at time of radical prostatectomy, compared to none in the group without cribriform architecture (P=0.006).

Comparison of invasive cribriform and intraductal carcinoma

Detailed histopathological and immunohistochemical analysis revealed that invasive cribriform carcinoma was present in 204 (48.6%), and intraductal carcinoma in 103 (24.5%) cases. Solid and loose papillary morphological variants of intraductal carcinoma were rarely observed, and co-existed with cribriform intraductal carcinoma in each case. Seventy-nine (18.8%) men had both intraductal carcinoma and invasive cribriform carcinoma, while 24 (5.7%) patients had intraductal carcinoma without invasive cribriform growth. Invasive cribriform growth without intraductal carcinoma was present in 125 men (29.8%). PSA levels (P=0.06), pT stage (P=0.32), surgical margin status (P=0.36) and occurrence of lymph node metastasis (P=0.39) were not significantly different between patients with invasive cribriform carcinoma without intraductal carcinoma

(n=125) and men with intraductal carcinoma only (n=24). Patients with both invasive cribriform and intraductal carcinoma (n=79) more frequently had extraprostatic extension (60.8% *versus* 46.4%; P=0.02) and lymph node metastasis (11.4% *versus* 1.6%; P=0.003) than those with invasive cribriform growth without intraductal carcinoma; there was no statistically significant difference in PSA level (P=0.07), pT stage (P=0.64), surgical margin status (P=0.20) and lymph node metastasis (P=0.32) between men with combined invasive cribriform and intraductal carcinoma, and intraductal carcinoma only.

Large invasive cribriform carcinoma

Large invasive cribriform growth was observed in 34 (8.1%) patients. All of these men (100%) had concomitant small invasive cribriform growth and 24 (70.6%) had intraductal carcinoma (Table 1.1). We compared patients with invasive large cribriform growth with men who had either small invasive cribriform growth and/ or intraductal carcinoma (n=194). The age of patients with large cribriform architecture (66.2 years; interquartile range 63.3-70.9) was higher (P=0.03) than of men with small cribriform architecture (63.8 years; interquartile range 60.1-67.8). Albeit PSA levels of men with large cribriform architecture were higher (15.0 ng/ml; interquartile range 8.3-18.3) than in those with small cribriform architecture (11.8 ng/ml; interquartile range 6.0-13.4), this did not reach significance in this cohort (P=0.16). In total 23/34 (67.7%) patients with large cribriform pattern had extraprostatic extension (pT3) as compared to 96/194 (49.0%) with small cribriform pattern (P<0.001), although positive surgical margins were more frequently observed in the latter group (23.5% *versus* 42.3%; P=0.04). The total percentage of Gleason grade 4 was 30.0% (interquartile range 20%-40%) in large and 23.3% (interquartile range 15%-30%) in small cribriform pattern (P=0.001). Tertiary Gleason grade 5 was observed in 8/34 (23.5%) patients with large and 23/194 (11.9%) with small cribriform architecture, but this difference did not reach conventional measures of significance (P=0.07). Lymph node metastases were observed in 6/26 (23.1%) men with large cribriform architecture and in 6/124 (4.8%) with small cribriform architecture (P=0.002).

Clinical outcome of invasive and intraductal carcinoma

The median follow-up of Grade Group 2 patients without positive lymph node dissection at time of radical prostatectomy (n=408) was 53 months (interquartile range 12.7-99.1). During follow-up 86 men experienced biochemical recurrence after a median of 26 (interquartile range 10.7-47.6) months. Biochemical recurrence occurred more frequently (χ^2, P=0.01) in the large invasive cribriform (13/28; 46.4%) than in the small invasive cribriform and/or intraductal group (44/188; 23.4%), and was lowest in Grade Group 2 patients without any cribriform growth (29/192; 15.1%,

P=0.04). The median time to biochemical recurrence was significantly shorter (log rank, P<0.001) in patients with large invasive cribriform growth (11 months; interquartile range 2.6-37.2) than in patients with small cribriform growth (25 months; interquartile range 11.3-39.3) and no cribriform architecture (43 months, interquartile range 15.4-73.8) (Figure 2.2).

Univariate analysis showed that PSA (hazard ratio 1.02, 95% CI 1.01-1.04; P=0.0001), pT3a (hazard ratio 2.00, 95% CI 1.27-3.14; P=0.003), pT3b (hazard ratio 4.42, 95% CI 2.24-8.72; P<0.001), positive surgical margins (hazard ratio 3.24, 95% CI 2.11-4.97; P<0.0001), intraductal carcinoma (hazard ratio 2.13, 95% CI 1.36-3.36; P=0.001) and any invasive cribriform growth (hazard ratio 1.78, 1.16-2.74; P=0.008) were all significant predictors for biochemical recurrence-free survival (Table 2.2). Percentage Gleason grade 4 was neither predictive as a continuous (hazard ratio 1.01, 95% CI 0.99-1.03, P=0.076) nor as a dichotomised parameter (hazard ratio 1.26, 95% CI 0.82-1.93, P=0.29). Tertiary Gleason grade 5 (hazard ratio 1.29, 95% CI 0.66-2.50, P=0.46) did not have predictive value for biochemical recurrence in this cohort. In multivariable analysis, extraprostatic extension (pT3a, hazard ratio 1.64, 95% CI 1.02-2.63, P=0.04), seminal vesicle invasion (pT3b, hazard ratio 3.00, 95% CI 1.42-6.34, P=0.004), positive surgical margins (hazard ratio 3.28, 95% CI 2.06-5.23, P<0.0001) and invasive large cribriform architecture (hazard ratio 4.36, 95% CI 2.08-9.17, P=0.0001) were independent predictors for biochemical recurrence-free survival, while small invasive cribriform growth pattern and intraductal carcinoma were not. To determine whether the difference in prognostic value between invasive small and large cribriform growth could be explained by an overall higher percentage of cribriform growth, we compared the outcome of patients with ≥5% invasive cribriform growth and those with <5%. When invasive cribriform growth was present, no statistical difference existed between low and high cribriform percentage (log rank; P=0.087).

During follow-up 13 patients developed bone metastases. Nine of these patients had small invasive cribriform or intraductal carcinoma (4.6%) and four had invasive large cribriform carcinoma (11.8%) at radical prostatectomy. The median time to bone metastasis was 138 months (interquartile range 109.4-172.6) for small invasive and intraductal cribriform carcinoma and 59 months (interquartile range 17.9-114.8) for invasive large cribriform carcinoma. Due to the low number of events we were not able to perform further statistical analysis.

Table 2.1. Clinicopathological characteristics of Grade Group 2 patients at radical prostatectomy. Non-cribriform cases do not have invasive cribriform carcinoma or intraductal carcinoma. Small cribriform cases include men with small invasive cribriform carcinoma and/or intraductal carcinoma. Large cribriform cases represent patients with presence of large invasive cribriform carcinoma, independent of the presence of small invasive cribriform carcinoma and intraductal carcinoma.

Characteristics	*Non-cribriform (n=192)*	*Small cribriform (n=194)*	*Large cribriform (n=34)*	*P-value*
Age at time of RP (years)	63.2 (64.0; 59.2-68.1)	63.8 (64.6; 60.1-67.8)	66.2 (67.0; 63.3-70.9)	0.03[1]
PSA level (ng/ml)	9.4 (7.7; 5.4-10.5)	11.8 (8.3; 6.0-13.4)	15.0 (11.5; 8.3-18.3)	0.16[1]
pT-stage (2009)				
T2	124 (64.6)	99 (51.0)	11 (32.4)	<0.001[2]
T3a	63 (32.8)	78 (40.2)	12 (35.3)	
T3b	5 (2.6)	17 (8.8)	11 (32.4)	
T4	0	0	0	
Positive surgical Margin	52 (27.1)	82 (42.3)	8 (23.5)	0.04[2]
Intraductal carcinoma	0	79 (40.7)	24 (70.6)	0.001[2]
IDC vs invasive cribriform				
IDC -/invasive cribriform -	192 (100.0)	0	0	<0.001[2]
IDC +/invasive cribriform -	0	24 (12.4)	0	
IDC -/invasive cribriform +	0	115 (59.3)	10 (29.4)	
IDC +/invasive cribriform +	0	55 (28.4)	24 (70.6)	
Tertiary Gleason 5	18 (9.4)	23 (11.9)	8 (23.5)	0.07[2]
PLND	91 (47.4)	124 (63.9)	26 (76.5)	0.16[2]
Lymph node metastasis	0	6 (4.8)	6 (23.1)	0.02[2]
Follow-up after RP (months)	62.6 (61.9; 14.6-100.2)	59.1 (41.3; 11.8-106.9)	53.2 (35.5; 13.2-73.4)	0.55[1]
BCR	29 (15.1)	48 (24.7)	18 (52.9)	0.001[2]
Distant metastasis	0	9 (4.6)	4 (11.8)	0.10[2]

Values denote either mean (median; interquartile range) or n (%).P-values correspond to the comparison between small invasive cribriform and/or intraductal carcinoma and large invasive cribriform groups.
[1]Student's t-test.
[2]Pearson's χ^2 test.

Figure 2.2. Biochemical recurrence-free survival of Grade Group 2 patients, stratified for absent, small invasive and/or intraductal carcinoma, and large invasive cribriform architecture growth (P-value <0.001).

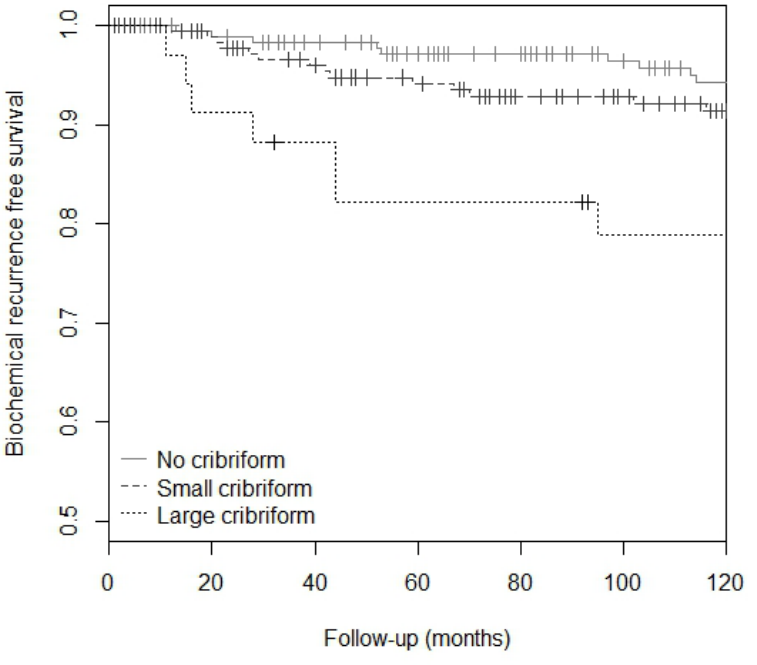

Table 2.2. Cox regression analysis of biochemical recurrence-free survival in Grade Group 2 prostate cancer patients without lymph node metastasis at time of operation (n=408).

	Univariate analysis			*Multivariable analysis*		
	HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Age	0.99	0.96 - 1.03	0.56	0.99	0.95 - 1.03	0.57
PSA	1.02	1.01 - 1.04	<0.001	1.01	0.99 - 1.02	0.34
pT-stage						
T2	*ref*			*ref*		
T3a	2.00	1.27 - 3.14	0.003	1.64	1.02 - 2.63	0.04
T3b	4.42	2.24 - 8.72	<0.001	3.00	1.42 - 6.34	0.004
Positive surgical margin	3.24	2.11 - 4.97	<0.001	3.28	2.06 - 5.23	<0.001
Percentage Gleason 4	1.26	0.82 - 1.93	0.29	0.94	0.59 - 1.51	0.80
Tertiary Gleason 5	1.29	0.66 - 2.50	0.46	0.95	0.44 - 2.06	0.90
Intraductal carcinoma	2.13	1.36 - 3.36	0.001	1.32	0.77 - 2.25	0.31
Invasive cribriform						
Small	1.50	0.95 - 2.37	0.09	1.07	0.65 - 1.75	0.80
Large	3.98	2.10 - 7.57	<0.001	4.36	2.08 - 9.17	<0.001

HR = hazard ratio, CI = confidence interval.

Discussion

While most patients with Grade Group 2 prostate cancer are treated with radiotherapy and/or surgery, active surveillance is increasingly being considered as alternative strategy for these men.[42-46] Further risk stratification in this large group of patients is necessary to support therapeutic decision-making. Recently, invasive cribriform carcinoma and intraductal carcinoma have been recognised as promising additional predictive parameters for men with Grade Group 2 prostate cancer.[14, 16, 32, 34, 47] In the current study, invasive and/or intraductal cribriform carcinoma was present in 54.3% of radical prostatectomies with Grade Group 2 prostate cancer. While the clinicopathological features of men with invasive cribriform carcinoma without cribriform intraductal carcinoma were not statistically significant from those with cribriform intraductal carcinoma only, patients with both invasive and intraductal cribriform carcinoma more often had extraprostatic extension and lymph node metastasis than those with invasive cribriform carcinoma only. Furthermore, we found that patients with large invasive cribriform growth had higher pT-stage and more frequent positive lymph nodes than those with small invasive and/or intraductal cribriform carcinoma. In multivariable analysis, large invasive cribriform carcinoma was an independent predictor for biochemical recurrence-free survival, while small invasive carcinoma and intraductal cribriform carcinoma were not.

Various studies have addressed the association of either invasive cribriform carcinoma or intraductal carcinoma with adverse features at prostatectomy and with clinical outcome. [14-16, 20, 34] We observed that invasive and intraductal cribriform carcinoma were present in respectively 48.6% and 24.5% of prostatectomy specimens with Grade Group 2 prostate cancer. These rates are comparable to those found by others. Trudel et al. for instance found intraductal carcinoma in 17.5%, invasive cribriform carcinoma in 45.6% and both invasive and intraductal cribriform carcinoma in 36.8% of 57 prostate specimens.[34] In a cohort of 286 Grade Group 2 prostate cancer patients, Choy et al. demonstrated intraductal carcinoma in 26.5% and invasive cribriform growth in 38.7%.[20] Two studies took into account large cribriform architecture, however these used different thresholds.[13, 34] Iczkowski et al. defined large cribriform pattern as having more than 12 luminal spaces, while area size exceeding the size of an average benign gland was used by Trudel et al. Our threshold of large cribriform fields as at least twice the size of normal adjacent glands, exceeds that of previous studies. For instance, in the study of Iczkowski et al. no cases were present with small cribriform pattern only, while small invasive cribriform carcinoma was present in 40% of our cases. To elucidate the clinical and biologic relevance of invasive cribriform and intraductal carcinoma in prostate cancer, it is crucial that it is clear how both entities are defined.

In a previous case-control study of 161 men with Gleason score 7 at radical prostatectomy, we found invasive cribriform but not intraductal carcinoma to be a significant predictive marker for metastasis- and disease specific-free survival in multivariate analysis.[16] In a subsequent analysis of prostate biopsies with long term follow-up, both invasive and intraductal carcinoma had predictive value for disease-specific death, and combining both lesions had the strongest prognostic value.[32] The prognostic value of invasive and intraductal carcinomas at biopsies does not always correspond with the prognostic value at radical prostatectomies. Sampling artifacts inherently associated with diagnostic biopsies are likely the cause of discrepancies between biopsies and radical prostatectomy specimens. This is for instance reflected by the frequency of cribriform growth in biopsies and resection specimens; while invasive and/or intraductal cribriform architecture was found in 17% of sextant biopsies with Grade Group 2, it was present in 54.3% of radical prostatectomy specimens in the current study.[32] Since most biopsy schedules currently include between 8 and 16 biopsies, and biopsies are increasingly being targeted by Magnetic Resonance Imaging (MRI), the frequency of invasive cribriform and/or intraductal carcinoma is higher with fewer sampling artifacts.[48, 49] Since both small cribriform growth and intraductal carcinoma are often associated with large cribriform growth, these patterns should still be reported.

The outcome of this study may have important implications. First, we propose the inclusion of the presence of large invasive cribriform in pathology reports. Of 26 men with large cribriform architecture who had undergone pelvic lymph node dissection at time of radical prostatectomy, 23% (n=6) had lymph node metastasis. Men with large cribriform architecture should therefore not be considered for surveillance but instead be offered active treatment with lymph node dissection. On the other hand, the absence of metastasis and low risk of biochemical recurrence in Grade Group 2 patients with no cribriform architecture might indicate that active surveillance can be considered in these men, and that pelvic lymph node dissection might be omitted when treatment is offered. However, it is important to note that the current results were obtained after studying radical prostatectomy specimens, while treatment decisions are made based on diagnostic biopsies. An urgent need exists to incorporate pathological features such as small and large invasive cribriform growth, as well as intraductal carcinoma, into clinical nomograms and prediction tools.

Strong points of this study are the detailed histological review and the extensive immunohistochemical staining for classification of cribriform architecture. Although large cribriform growth is an adverse predictive parameter for Grade Group 2 prostate cancer patients, the stringent cut-off used in this study resulted in the inclusion of a relatively small number of cases

and must be validated. Finally, the retrospective study design and relatively short median follow-up of 53 months possibly gave rise to a selection bias.

In conclusion, we demonstrate that patients with large invasive cribriform growth represent a more aggressive subgroup of cribriform Grade Group 2 prostate cancer. Men with large invasive cribriform carcinoma should be actively treated since they are at increased risk for biochemical recurrence and metastasis.

Chapter III

Concordance of cribriform architecture in matched prostate cancer biopsy and radical prostatectomies.

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Histopathology. 2019 Sept; 75: 338-345.

Abstract

Invasive cribriform and/or intraductal carcinoma have been identified as independent adverse parameters for prostate cancer outcome. Little is known on biopsy undersampling of cribriform architecture. Our aim was to determine the extent of cribriform architecture undersampling and to find predictive factors for identifying false cribriform negative cases.

We reviewed 186 matched prostate biopsies and radical prostatectomy specimens. Of 97 biopsy Grade Group 2 (Gleason score 3+4=7) patients, 22 (23%) had true cribriform negative (TN), 39 (40%) false negative (FN) and 36 (37%) true positive (TP) biopsies. Patients with FN biopsies had higher albeit not statistically significant (P=0.06) median PSA levels than patients with TP biopsies (12 *versus* 8 ng/ml). A PI-RADS 5 lesion was present in 9/16 (54%) FN and 3/11 (27%) TN biopsies (P=0.05). Positive biopsy rate (P=0.47), percentage Gleason pattern 4 (P=0.55) and glomeruloid architecture (P=1.0) were not different. Logistic regression identified PSA as independent predictor (Odds Ratio 3.5; 95% Confidence Interval 1.2-9.4, P=0.02) for cribriform architecture on radical prostatectomy but not PI-RADS score. The FN rate for large cribriform architecture at radical prostatectomy was 27%, which was lower than for any cribriform architecture (P=0.01). During follow-up (median 27 months), biochemical recurrence-free survival of patients with TP biopsies was significantly shorter than of those with FN biopsies (P=0.03).

In conclusion, 40% of Grade Group 2 prostate cancer biopsies were FN for cribriform architecture. These patients had higher PSA levels and more frequent PI-RADS score 5 lesions than men with TN biopsies.

Introduction

Risk stratification and therapeutic decision-making in prostate cancer patients is affected by potential biopsy undersampling. The Gleason score is one of the most important parameters for predicting disease outcome and guiding individual treatment. Men with Gleason score 3+3=6 (International Society of Urological Pathology (ISUP) Grade Group 1) prostate cancer are eligible for active surveillance, whereas men with Gleason score ≥ 4+3=7 (Grade Group 3-5) are usually treated with radical prostatectomy, radiation therapy and/or hormonal therapy. The optimal therapeutic strategy for men with Gleason score 3+4=7 (Grade Group 2) still is a matter of debate. While most of these patients will undergo active treatment, surveillance is increasingly being considered in this subgroup. Incorporation of additional clinicopathological and molecular parameters might be able to support optimal decision-making in this large prostate cancer subpopulation.

Grade Group 2 prostate cancer is a heterogeneous disease with variable architectural growth patterns and Gleason pattern 4 quantities. While individual growth patterns are not routinely mentioned in pathology reports, recent studies have shown that patients with cribriform architecture have adverse outcome as compared to those without.[15, 39, 50] Both invasive and intraductal cribriform architecture have been associated with adverse clinicopathological characteristics, post-operative recurrence rates, metastasis and disease-specific death.[14, 16, 32, 34, 51] On the other hand, biopsy Grade Group 2 prostate cancer patients without cribriform architecture have comparable disease-specific survival and post-operative biochemical recurrence rates as men with Grade Group 1 disease.[39, 52] Quantification of Gleason pattern 4 can further add in risk stratification since post-operative biochemical recurrence rates increment with higher Gleason pattern 4 tumour percentage.[53] Cribriform architecture and Gleason pattern 4 quantification might therefore be important adjuncts in risk stratification of Grade Group 2 prostate cancer patients.

While pathological tumour characteristics are important for clinical decision-making, prostate biopsies are prone to undersampling. Prostate cancer is upgraded in up to 40% of subsequent radical prostatectomy specimens.[54, 55] At present, little is known on the extent of undersampling in detection of cribriform architecture or Gleason pattern 4 percentage. The aim of our study is to determine the extent of undersampling for the detection of cribriform architecture in matched prostate biopsy and radical prostatectomy specimens, and to identify potential factors for discriminating true from false cribriform negative prostate biopsies.

Materials and Methods

Patient selection

We identified 186 patients who had undergone both biopsy and subsequent radical prostatectomy at Erasmus MC University Medical Center, Rotterdam, The Netherlands between 2010 and 2017. Biopsies were prompted by elevated Prostate Specific Antigen (PSA) levels or obtained in the scope of active surveillance. The Prostate Imaging Reporting and Data System (PI-RADS) score was annotated by an expert uroradiologist, when patients had received multiparametric magnetic resonance imaging (MRI).[56] When suspicious lesions (PI-RADS 3 to 5) were visible on MRI, targeted MRI-ultrasound fusion biopsies were taken. Individual biopsy cores were enclosed in separate containers and radical prostatectomy specimens were completely embedded for diagnostic purposes. All slides of both biopsies and radical prostatectomies were available for pathologic review. This study was approved by the institutional Medical Research Ethics Committee (MEC-2018-1614).

Pathologic evaluation

All biopsies were reviewed by three investigators, who were blinded to clinical outcome and radical prostatectomy characteristics. For each biopsy core the following features were recorded: Gleason score, Grade Groups according to the WHO/ISUP 2014 guidelines, maximal single biopsy tumour length (mm), overall estimated percentage Gleason pattern 4 and individual tumour growth patterns.[12] Invasive cribriform Gleason pattern 4 was not distinguished from intraductal carcinoma because of their significant morphological overlap, which would require extensive immunohistochemical staining for further discrimination.[39] In case targeted biopsies were obtained, these were considered as separate biopsies and not as one single biopsy. Matching radical prostatectomy specimens were evaluated as described previously.[51] We recorded Gleason score, Grade Group, pT-stage according to the AJCC TNM 8th edition, surgical margin status, percentage Gleason pattern 4 and individual growth patterns.[41] Furthermore, we distinguished small and large expansive cribriform growth pattern based on a cut-off of two times the size of adjacent pre-existent normal glands.[51]

Clinical follow-up

After radical prostatectomy, clinical follow-up consisted of bi-annual, and later annual monitoring of serum PSA levels. Biochemical recurrence was defined as PSA levels $\geq$ 0.2 ng/ml measured at two consecutive points in time, at least three months apart with undetectable PSA levels after operation, or as PSA increase of > 2.0 ng/ml when serum PSA had not declined to zero after

operation. Survival was defined as time in months from radical prostatectomy to biochemical recurrence or last follow-up.

Statistical analysis

Continuous variables with normal distribution were compared by Student's t-test and One-way ANOVA analysis, those without normal distribution with the Mann-Whitney U test. For categorical parameters Chi-square or Fishers exact were used. Correlation between continuous variables was analysed using Pearson's correlation coefficient. Dichotomous outcome variables were analysed using logistic regression. Survival was visualised by Kaplan-Meier curves. Statistics were performed using R version 3.2.2 (R, Vienna, Austria) and results were considered significant when the two-sided P-value was <0.05.

Results

Clinicopathological characteristics

The entire cohort consisted of 186 patients with matched biopsy and radical prostatectomy specimens. The mean age at time of operation was 65 years (interquartile range (IQR) 62-70) and the mean PSA level was 12 ng/ml (IQR 6-15). In total 144 (77%) patients underwent systematic biopsies, 26 (14%) received systematic and targeted biopsies, and 16 (9%) had targeted biopsies only. The mean number of biopsies taken was 9 (IQR 8-10) with 4 (IQR 3-5) biopsies containing adenocarcinoma, representing 49% (IQR 30-66) of the total number of biopsy cores. Fifty (27%) patients had overall biopsy Grade Group 1, 99 (53%) Grade Group 2, 11 (6%) Grade Group 3, 15 (8%) Grade Group 4 and 11 (6%) Grade Group 5.

On radical prostatectomy, 87 (47%) adenocarcinomas were pT2, 76 (41%) pT3a and 23 (12%) pT3b. Distribution of the Grade Groups on radical prostatectomy was as follows: 19 (10%) Grade Group 1, 108 (58%) Grade Group 2, 25 (14%) Grade Group 3, 17 (9%) Grade Group 4 and 17 (9%) Grade Group 5. Tumour upgrading occurred in 65 (35%) and down-grading in 14 (8%) radical prostatectomies, while 107 (57%) cases had concordant tumour grades. Positive surgical margins were present in 63 (34%) patients. Eighty patients had simultaneously undergone pelvic lymph node dissection, of which 18 (23%) contained lymph node metastasis. The mean post-operative follow-up was 32 months (median 22, IQR 8-51).

Invasive cribriform and/or intraductal carcinoma was observed in 57 (31%) diagnostic biopsies and in 128 (69%) radical prostatectomy specimens (Table 3.1). Cribriform architecture was present in both matched biopsy and radical prostatectomy specimens in 55 (30%), and absent in 56 (30%) cases. In 73 (39%) men cribriform architecture was observed in the radical prostatectomy specimen, but not in preceding biopsies. Two cases (1%) with cribriform architecture at biopsy but not at subsequent radical prostatectomy, probably due to sampling error, were excluded from further analyses. Therefore, sensitivity for cribriform architecture on biopsies was 43%, while specificity was 97%. Cribriform architecture was observed more frequently in targeted (19/40; 48%) than systematic biopsies (36/144; 25%, P=0.01).

Table 3.1. Prevalence of invasive cribriform and/or intraductal carcinoma (CR/IDC) in biopsies and matched radical prostatectomies.

	Radical prostatectomy	
Prostate biopsy	CR/IDC-	CR/IDC+
CR/IDC-	56 (30%)	73 (39%)
CR/IDC+	2 (1%)	55 (30%)

Concordance of cribriform architecture in Grade Group 2 prostate cancer biopsies

Since cribriform architecture might be most relevant for treatment decisions in patients with biopsy Grade Group 2 prostate cancer, we performed further analyses within this subgroup (n=97). Thirty six (37%) patients with biopsy Grade Group 2 demonstrated cribriform architecture on both matched biopsy and radical prostatectomy specimen (true cribriform positive, CR+/CR+), while cribriform architecture was absent in both specimens in 22 (23%) cases (true cribriform negative, CR-/CR-). In 39 (40%) patients cribriform architecture was present on radical prostatectomy but not on preceding biopsy; these patients were considered as having false cribriform negative (CR-/CR+) biopsies. None of the patients with biopsy Grade Group 2 had cribriform architecture on biopsy while radical prostatectomy was negative for cribriform architecture.

Identification of predictors in true and false cribriform negative Grade Group 2 prostate cancer biopsies

Patients with true negative biopsies were slightly younger (62 *versus* 65 years, P=0.06) and had lower PSA levels (8 ng/ml *versus* 12 ng/ml, P=0.06) than men with false negative biopsies, however these differences were not significant (Table 3.2). In total, 51 patients (53%) had undergone multiparametric MRI prior to biopsy. Out of 11 patients with true negative biopsies, 3 (27%) had a PI-RADS 5 lesion as compared to 9/16 (56%) of false negative and 17/24 (71%) of true positive biopsy patients (P=0.05). The number of biopsies (P=0.53), percentage of positive biopsies (P=0.47) and maximal tumour length (P=0.44) were not different between true and false negative biopsies.

Since Gleason pattern 4 percentage and glomeruloid architecture have both been associated with cribriform architecture, we assessed the predictive value of these pathologic parameters.[16, 19] Mean percentage of Gleason pattern 4 was 12% (IQR 5-10%) in true negative biopsies and 11% (IQR 5-16%) in false negative biopsies (P=0.55). There was only a weak correlation between percentage Gleason pattern 4 on biopsies (mean 13%, IQR 5-20%) and matched radical prostatectomies (mean 31%, IQR 10-40%, R^2=0.093; P=0.001). Glomeruloid growth pattern was encountered in 6/22 (27%) true negative and 11/39 (28%) false negative biopsies (P=1.0).

Logistic regression analysis on cribriform negative biopsy patients showed that age (odds ratio (OR) 1.1, 95% confidence interval (CI) 1.0-1.3, P=0.02) and PSA (OR 3.3, 95% CI 1.2-9.1, P=0.02) were independent predictive parameters for presence of cribriform architecture on radical prostatectomy in multivariable analysis, whereas PI-RADS score, number and percentage of positive biopsies, maximal tumour length, presence of targeted biopsies and percentage Gleason grade 4 were not (Table 3.3).

Table 3.2. Characteristics of biopsy Grade Group 2 prostate cancer (PCa) patients stratified for true cribriform negative (CR-/CR-), false cribriform negative (CR-/CR+) and true cribriform positive (CR+/CR+) biopsies.

	CR-/CR- (n=22)	CR-/CR+ (n=39)	CR+/CR+ (n=36)	P-value
Age	62 (63, 58-65)	65 (66, 62-71)	66 (66, 62-71)	0.06[a]
PSA	8 (8, 6-10)	12 (10, 6-17)	16 (13, 9-19)	0.06[b]
PI-RADS score: no MRI	11 (50%)	23 (59%)	12 (33%)	0.10[c]
1-2	3 (14%)	0 (0%)	0 (0%)	
3	1 (5%)	1 (3%)	2 (6%)	
4	4 (18%)	6 (15%)	5 (14%)	
5	3 (14%)	9 (23%)	17 (47%)	
Number of biopsies	9 (9, 8-10)	8 (8, 7-10)	10 (10, 8-12)	0.53[d]
# PCa positive biopsies	4 (3, 2-6)	4 (4, 3-5)	6 (5, 4-8)	0.64[d]
% PCa positive biopsies	47 (38, 25-71)	52 (50, 31-73)	59 (61, 40-76)	0.47[d]
Max tumour length (mm)	7 (7, 5-8)	8 (7, 5-10)	9 (10, 7-12)	0.44[d]
% Gleason pattern 4	12 (8, 5-10)	11 (8, 5-16)	17 (15, 7-23)	0.55[a]
Glomeruloid growth	6 (27%)	11 (28%)	12 (33%)	1.0[e]
Large cribriform growth	0	6 (15%)	16 (44%)	N/A
Targeted biopsies	2 (9%)	8 (20%)	13 (36%)	0.30[e]
Grade Group (RP): 1	2 (9%)	1 (3%)	1 (3%)	0.01[e]
2	18 (82%)	29 (74%)	26 (72%)	
3	0 (0%)	8 (20%)	7 (19%)	
4	0 (0%)	1 (3%)	1 (3%)	
5	2 (9%)	0 (0%)	1 (3%)	
Positive surgical margins	8 (36%)	12 (31%)	12 (33%)	0.78[c]
pT stage (TNM 8th): 2	11 (50%)	15 (38%)	17 (47%)	0.66[c]
3a	10 (45%)	20 (51%)	12 (33%)	
3b	1 (5%)	4 (11%)	7 (20%)	
Biochemical recurrence	2 (9%)	6 (15%)	13 (36%)	0.69[e]
Metastasis	0 (0%)	1 (3%)	4 (11%)	N/A

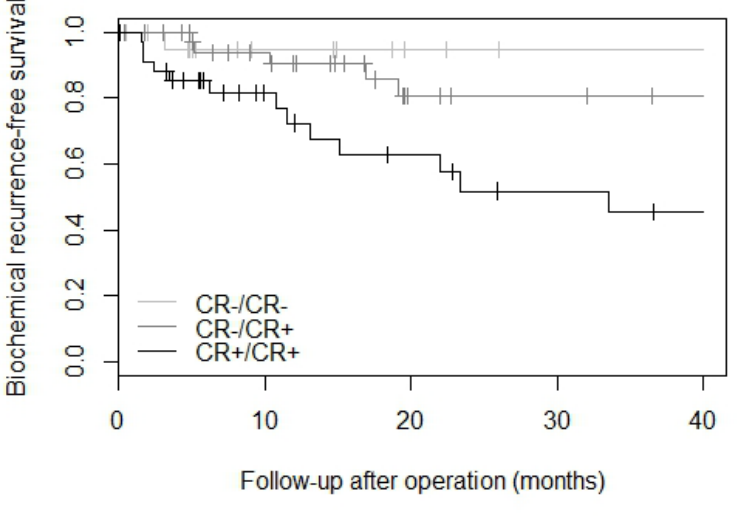
Mean (median, IQR) or n (%). [a] Wilcox-test, [b] t-test (log2 values were used for this test), [c] Chi-square (χ^2), [d] One-Way Anova, [e] Fisher test. P-values resemble comparison between CR-/CR- and CR-/CR+.

Table 3.3. Logistic regression analysis of biopsy Grade Group 2 cribriform negative prostate cancer (PCa) patients (n=61), predicting cribriform architecture on radical prostatectomy.

	Univariate			Multivariable		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.1	1.0-1.2	0.06	1.1	1.0-1.3	0.02
PSA (log2)	2.2[a]	1.0-4.8	0.04	3.3[a]	1.2-9.1	0.02
PI-RADS score						
<5	*ref*					
5	1.9	0.5-7.9	0.38	1.8	0.3-9.1	0.49
Number of biopsies	0.9	0.8-1.1	0.53	0.8	0.6-1.1	0.21
Percentage PCa positive biopsies	2.1	0.3-15	0.47	0.2	0.0-5.5	0.35
Maximal tumour length (mm)	1.1	0.9-1.2	0.43	1.0	0.9-1.3	0.70
Percentage Gleason pattern 4	1.0	0.9-1.0	0.70	1.0	0.9-1.0	0.36
Presence of targeted biopsies						
No	*ref*					
Yes	2.6	0.5-13	0.26	1.1	0.1-10	0.91

[a] Per doubling unit. OR = odds ratio, CI = confidence interval.

Figure 3.1. Biochemical recurrence-free survival of biopsy Grade Group 2 prostate cancer patients, stratified for the presence of cribriform architecture on biopsies and subsequent radical prostatectomies (log rank over all groups, P-value = 0.02).



Comparison of false negative and true cribriform positive Grade Group 2 biopsies

PSA levels of men with true positive biopsies were slightly higher than of those with false negative biopsies, but this was not statistically significant (16 ng/ml *versus* 12 ng/ml, P=0.13). Patients with true positive biopsies had a significantly higher total number of biopsies (10 *versus* 8, P=0.02) and number of tumour positive biopsies (6 *versus* 4, P=0.001), however no differences were seen in percentage positive biopsies (59% *versus* 52%, P=0.19) when compared to patients with false negative biopsies. Percentage Gleason pattern 4 was higher in patients with cribriform positive biopsies than in those with false negative biopsies (17% *versus* 11%, P=0.03). Final Grade Group (P=0.97), pT stage (P=0.27) and surgical margin status (P=0.24) of the radical prostatectomy specimens were not different between these two groups. The tumour volume percentage of cribriform growth at radical prostatectomy was higher in patients with true positive biopsies than in those with false negative biopsies, but this did not meet conventional measures of significance (13% *versus* 6%, P=0.06).

Large expansile cribriform architecture, which represents an aggressive subtype of invasive cribriform carcinoma, was present in 22/97 (23%) radical prostatectomy specimens.[51] Sixteen of these 22 (73%) patients had any size cribriform fields on biopsy, while biopsies were false negative in 6 (27%) men. The false negative rate for more aggressive large cribriform architecture (6/22; 27%) was lower than for any cribriform architecture (39/75; 52%, P=0.01). In case large cribriform carcinoma was present at radical prostatectomy, the tumour volume percentage of any cribriform growth at the operation specimens did not differ between men with false cribriform negative and true positive biopsies (P=0.5). This indicates that the lower false negative rate of large cribriform growth was not merely due to larger total cribriform tumour percentage at radical prostatectomy.

Clinicopathological outcome in Grade Group 2 patients

Of 97 patients with biopsy Grade Group 2 prostate cancer, 73 (75%) had concordant Grade Group at radical prostatectomy, 20 (21%) were upgraded to Grade Group 3 to 5, and 4 (4%) down-graded to Grade Group 1. Upgrading occurred in 9/36 (25%) true positive and in 9/39 (23%) false negative biopsies, and was significantly lower (P=0.01) in true negative biopsies (2/22, 9%). Extra-prostatic expansion and surgical margins status were not significantly different between the three groups.

Biochemical recurrence occurred in 21 (22%) patients and was significantly more frequent in the true positive (13/36, 36%) than in the false negative group (6/39, 15%, P=0.03). The true negative group (2/22, 9%) showed the lowest incidence of biochemical recurrence, however this difference was not significant (P=0.13) when compared to the false negative group.

The median post-operative follow-up of Grade Group 2 patients was 27 months (mean 18, IQR 6-40). Patients experienced biochemical recurrence after a median of 14 months (mean 24, IQR 5-32). Biochemical recurrence-free survival was not significantly different between patients with true negative and false negative biopsies (log rank P=0.55). Patients with cribriform positive biopsies had significantly shorter biochemical recurrence-free survival than men with false negative biopsies (log rank P=0.03, Figure 1).

Discussion

Identification and pathologic reporting of invasive cribriform and/or intraductal carcinoma of the prostate are increasingly important since they are both associated with adverse clinical outcome.[14, 34, 39, 50] Biopsy undersampling is a well-known problem which might have significant impact on individual patient management.[54, 57, 58] Hitherto, little is known about biopsy undersampling in identifying cribriform architecture. In this study we demonstrated that biopsies were false negative for cribriform architecture in 39% of all cases and in 40% of patients with biopsy Grade Group 2 prostate cancer. In false negative Grade Group 2 patients, age and PSA level were independent predictive parameters for presence of cribriform architecture on subsequent radical prostatectomy, while percentage of positive biopsies, maximal biopsy tumour length, percentage Gleason pattern 4 and glomeruloid growth were not. Patients with the more aggressive large cribriform growth pattern on radical prostatectomy were, however, less likely to have cribriform negative biopsies.[51] Biopsy Grade Group 2 patients with false cribriform negative biopsies showed better biochemical recurrence-free survival rates than men with true cribriform positive biopsies albeit follow-up was relatively short.

Masoomian et al. studied concordance rates of cribriform architecture in 245 matched biopsies and operation specimens, and found a relatively low sensitivity of 47%, corresponding well with the 43% sensitivity in our study.[59] In their subset of Grade Group 2 biopsy patients, false negative and true positive biopsies both had more advanced stage as compared to true negative biopsies on radical prostatectomy suggesting men with false negative and true positive biopsies have comparable outcome. This contrasts with our study as we found that post-operative biochemical recurrence-free survival of men with true positive biopsies was significantly shorter than of those with false negative biopsies. The difference might be explained by the different and relatively small cohorts of both studies.

While most patients with biopsy Grade Group 2 prostate cancer undergo active treatment, the question is rising whether surveillance could be a safe alternative for subgroups of this large patient population. It has for instance been proposed that patients with biopsy Grade Group 2 prostate cancer and low Gleason pattern 4 percentage should be considered for surveillance.[44, 60] Others have suggested that biopsy Grade Group 2 prostate cancer patients without invasive cribriform and/or intraductal carcinoma might be eligible for surveillance.[32, 52] To further support clinical decision tools, it is important to get insight in the false negative rate of potentially aggressive disease parameters and to determine how this rate can be minimised to an acceptable level. In the current study, we showed that consideration of PSA level, which is an important parameter for active surveillance, might prevent men with potentially aggressive false

negative biopsies from being abstained from immediate treatment. Furthermore, presence of a PI-RADS 5 lesion on multiparametric MRI might also be indicative of more aggressive disease. Truong et al. identified cribriform morphology in combined systematic and targeted biopsies in 37% of PI-RADS 5, 24% of PI-RADS 4 and 6% of PI-RADS 2 lesions, suggesting that high-grade MRI lesions are related to more aggressive tumours with cribriform morphology.[48] Prendeville et al. identified cribriform morphology in 8% of PI-RADS 3/4 lesions and in 39% of PI-RADS 5 lesions, indicating that PI-RADS score might be a predictor for cribriform positive prostate cancer.[49] Here we showed that 56% of false negative biopsies had a PI-RADS 5 lesion as compared to 27% of true negative biopsies. However, due to the small number of patients that had undergone MRI, PI-RADS score was not a predictor for cribriform architecture in logistic regression analysis.

We were not able to find any predictive value of biopsy percentage Gleason pattern 4 or glomeruloid growth pattern for cribriform architecture on radical prostatectomy. Presence of cribriform architecture has been associated with higher percentage Gleason pattern 4 on biopsies. In a cohort of 370 biopsy Grade Group 2 prostate cancer patients, we found cribriform architecture in 6% of men with <10% Gleason pattern 4, in 22% of men with 10-25% pattern 4, and in 44% of men with 25-50% pattern 4.[32] Nevertheless, biopsy percentage Gleason pattern 4 was not predictive for cribriform architecture in false negative biopsies. This paradoxical outcome could be explained by the low level of concordance between percentage Gleason pattern 4 on biopsy and matched radical prostatectomy specimens in this study. Similarly, glomeruloid Gleason pattern 4 which has been hypothesised to represent a precursor lesion of cribriform growth, was not associated with cribriform architecture in false negative biopsies.[19]

Amongst patients with cribriform architecture, those with large expansive cribriform fields have the worst outcome.[51] The false negative rate of 27% for large cribriform pattern is significantly less than the rate of 52% for overall cribriform morphology. Since 44% of true positive biopsies had large cribriform fields on radical prostatectomy as compared to only 15% of false negative biopsies, this might explain the significantly better biochemical recurrence-free survival of false negative biopsies as compared to true positive biopsies, in addition to other clinicopathological confounding factors.

The strong points of this study are the detailed histological review of matched biopsies and radical prostatectomies. The study is however limited by its low number of patients, the heterogeneity of the study population including both patients with first-time diagnosis and progression during active surveillance, and variability of diagnostic work-up encompassing systematic and/or targeted biopsies as well as multiparametric MRI assessment. Finally, follow-up is relatively short with a median of 27 months.

In conclusion, we demonstrate that 40% of men with biopsy Grade Group 2 prostate cancer were false negative for invasive cribriform and/or intraductal carcinoma. Age and PSA were independent predictors for cribriform architecture in false negative biopsies, while patients with false negative biopsies more frequently had PI-RADS score 5 lesions than men with true negative biopsies. Multimodal evaluation of biopsy Grade Group 2 prostate cancer patients could therefore identify men with true cribriform negative biopsies who might become eligible for active surveillance.

Chapter IV

Clinical outcome comparison of Grade Group 1 and Grade Group 2 prostate cancer with and without cribriform architecture.

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Histopathology. 2020 Apr; 76: 755-762.

Abstract

Invasive cribriform and intraductal carcinoma are associated with aggressive disease in Grade Group 2 (GG2) prostate cancer patients. However, the characteristics and clinical outcome of Grade Group 2 patients without cribriform architecture (GG2-) compared to those with Grade Group 1 (GG1) disease are unknown. The aim of this study was to investigate the clinical and pathological characteristics of GG1 and GG2- prostate cancer in radical prostatectomy specimens. We reviewed 835 radical prostatectomy specimens for Grade Group, pT-stage, surgical margin status and presence of cribriform architecture. Biochemical recurrence-free survival and metastasis were used as clinical outcomes. GG1 prostate cancer was seen in 207 and GG2 in 420 patients, of whom 228 (54%) showed cribriform architecture (GG2+) and 192 (46%) did not. Patients with GG2- disease had higher Prostate Specific Antigen levels (9.4 *versus* 7.0 ng/ml; P<0.001), more often extra-prostatic extension (36% *versus* 11%; P<0.001) and more frequent positive surgical margins (27% *versus* 17%; P=0.01) than those with GG1. GG2- patients had shorter biochemical recurrence-free survival (Hazard Ratio (HR) 2.7, 95% Confidence Interval (CI) 1.4-4.9; P=0.002) than those with GG1. Lymph node and distant metastasis were neither observed in GG2- nor in GG1 patients, but occurred in 22/228 (10%) of GG2+ patients.

In conclusion, patients with GG2- prostate cancer at radical prostatectomy have more advanced disease and shorter biochemical recurrence-free survival than men with GG1, but both groups have very low risk of developing metastasis.

Introduction

Active surveillance is increasingly applied in men with prostate cancer. Whereas most men with biopsy Grade Group 1 (Gleason score 3+3=6, GG1) prostate cancer are eligible for active surveillance, inclusion of favourable Grade Group 2 (Gleason score 3+4=7, GG2) patients with limited Gleason pattern 4 is gradually accepted.[45, 61-64] In general, these patients have Prostate Specific Antigen (PSA) levels of <10 ng/ml, present with organ-confined disease and have <10% Gleason pattern 4 in their diagnostic biopsies.[7]

Gleason pattern 4 prostate cancer is a heterogeneous disease encompassing various histopathological growth patterns. Invasive and/or intraductal cribriform carcinoma, both also referred to as cribriform architecture, have been identified as pathological parameters for worse outcome in both biopsy as well as radical prostatectomy specimens.[13-17, 34] Cribriform architecture has been associated with advanced tumour stage, biochemical recurrence, metastasis and disease-specific death in GG2 prostate cancer.[32, 50, 51] While patients with GG2 prostate cancer without cribriform architecture (GG2-) have favourable outcome compared to those with invasive and/or intraductal cribriform carcinoma (GG2+), it is unclear to what extent GG2- differs from GG1 disease.

In previous sextant biopsy studies with long-term follow-up, patients with biopsy GG2- prostate cancer had similar biochemical recurrence-free and disease-specific survival as men with GG1 disease.[39, 52] Therefore, it has been proposed that patients without cribriform architecture might be eligible for active surveillance.[32, 39, 44, 52, 60, 65] Prostate biopsies are, however, subject to significant sampling errors with tumour undergrading in up to 40% and there is low sensitivity for detection of cribriform architecture.[58, 59, 66] Moreover, in contrast to radical prostatectomy specimens, minor high-grade patterns are always taken into account when grading prostate cancer biopsies. To elucidate on its clinical and biological features, GG2- prostate cancer should be investigated on radical prostatectomy specimens, which excludes study bias by biopsy sampling artefacts. The aim of this study was to compare the clinicopathological characteristics and biochemical recurrence-free survival of GG1 and GG2- prostate cancer patients in radical prostatectomy specimens.

Materials and methods

Patient selection

In total, 854 men who had undergone radical prostatectomy for prostate adenocarcinoma at Erasmus MC, University Medical Center, Rotterdam, The Netherlands between 2000 and 2017 were included. Men who had received hormonal, radiation or viral therapy (n=19) prior to operation were excluded from this study.[40] After fixation in neutral-buffered formalin, radical prostatectomy specimens were sectioned transversely and totally embedded for diagnostic purposes. All slides were available for pathology review. The use of tissue samples for scientific purposes was approved by the institutional Medical Research Ethics Committee (MEC-2018-1614).

Pathologic evaluation

All 835 radical prostatectomy specimens were reviewed by two investigators (EH, GvL), who were blinded to clinical outcome. The following features were recorded: Gleason score and Grade Group according to the World Health Organization (WHO) 2016 guidelines, pT-stage according to the American Joint Committee on cancer (AJCC) TNM 8th edition, surgical margin status, Gleason pattern 3 to 5 percentages, Gleason 4 growth patterns and presence of intraductal carcinoma.[12, 38, 41] Intraductal carcinoma was not incorporated in the Gleason score. Tertiary Gleason patterns occupied less than 5% of the total tumour area and were not incorporated in the Gleason score.[12, 38]

In order to most accurately distinguish intraductal form invasive cribriform carcinoma, the following criteria were used. Invasive cribriform Gleason grade 4 was morphologically distinguished from intraductal carcinoma when it had an irregular outline, anastomosing fields beyond pre-existent gland architecture or extension into periprostatic fat tissue, ejaculatory ducts or seminal vesicles. Intraductal carcinoma was morphologically identified if cribriform structures were clearly continuous with pre-existent glands lined by normal basal epithelium, or containing corpora amylacea. When invasive cribriform carcinoma and intraductal carcinoma could not be differentiated by morphological criteria alone, additional immunohistochemical staining for the presence of basal cells was performed.

Immunohistochemistry

Four micrometer thick tissue sections were cut from selected paraffin-embedded blocks (Superfrost Microscopic Slides, ThermoFisher Scientific, Bleiswijk). Slides were deparaffinized and rehydrated with xylene and ethanol. Endogenous peroxidase was blocked using 0.3% H_2O_2 in PBS

and heat-induced antigen retrieval accomplished by 15 min in Tris-EDTA buffer (pH 9; Klinipath, Duiven, The Netherlands). Mouse monoclonal high molecular weight cytokeratin (clone 34BE12; 1:200; DAKO; Heverlee, Belgium) diluted in normal antibody diluent (APG-500; ScyTek Laboratories, West Logan, USA) was incubated for 2 hours at room temperature. Antibody visualization was performed using the Envision kit (DAKO) and slide counterstaining with hematoxylin. When basal cell staining was absent, the cribriform structure was categorized as invasive carcinoma; if sporadic, scattered or continuous basal cells were identified the growth pattern was classified as intraductal carcinoma.

Clinical follow-up

Clinical follow-up after radical prostatectomy consisted of six-monthly, and later annual monitoring of serum PSA levels. Biochemical recurrence was defined as PSA level ≥ 0.2 ng/ml measured at two separate points in time at least three months apart when PSA had been undetectable after operation, or as PSA increase of > 2.0 ng/ml whenever serum PSA had not declined to zero after operation. Post-operative lymph node and distant metastases were confirmed by biopsy or multidisciplinary consensus. Biochemical recurrence-free survival was defined as time in months from radical prostatectomy to biochemical recurrence.

Statistical analysis

Normally distributed, continuous variables were analysed using the independent sample Student's t-test. Pearson's chi squared (χ^2) test was used for categorical parameters. Missing PSA values (n=27) were imputed using the median PSA value. Biochemical recurrence-free survival was analysed using Cox proportional hazards regression and visualized by Kaplan-Meier curves. Statistics were performed using SPSS version 24 (IBM, Chicago, IL, USA). Results were considered significant when the two-sided P-value was <0.05.

Results

Patient characteristics

Out of 835 radical prostatectomy specimens, 207 had GG1 and 420 GG2 prostate cancer. The median age of these 627 patients at time of operation was 64.1 years (interquartile range (IQR) 59.8-67.6 years) and the median PSA level was 7.6 ng/ml (IQR 5.4-10.8 ng/ml). Pathologic tumour stage was distributed as follows: 419 (66%) pT2, 173 (28%) pT3a and 35 (6%) pT3b tumours. Positive surgical margins were present in 177 (28%) cases. Pelvic lymph node dissection was performed in 375 (60%) men, of whom 12 (3%) had lymph node metastasis.

Invasive cribriform and/or intraductal carcinoma

Among men with GG2 prostate cancer, 228 (54%) had invasive cribriform and/or intraductal carcinoma (GG2+) and 192 (46%) did not (GG2-). GG2+ patients had higher PSA levels (12.2 ng/ml *versus* 9.4 ng/ml; P=0.006), higher percentage Gleason pattern 4 (24% *versus* 18%; P<0.001), more frequent extra-prostatic extension (pT3; 52% *versus* 36%; P<0.001), positive surgical margins (40% *versus* 27%; P=0.007) and lymph node metastases (8% *versus* 0%; P=0.001) than GG2- patients. Patients with GG2- presented with higher median PSA levels (9.4 *versus* 7.0 ng/ml; P<0.001), more frequent extra-prostatic extension (36% *versus* 11%; P<0.001) and positive surgical margins (27% *versus* 17%; P=0.01) than men with GG1 prostate cancer (Table 4.1). None of the patients with GG1 or GG2- tumours had metastasis at lymph node dissection.

Tertiary Gleason pattern 4 in Grade Group 1 prostate cancer

GG1 prostate cancer in radical prostatectomy specimens might by definition contain tertiary high-grade patterns. To investigate to what extent GG2- disease differed from GG1 with tertiary pattern and/or pure GG1, we analysed both GG1 subgroups separately. Tertiary Gleason pattern 4 was present in 42 out of 207 (20%) GG1 patients, of whom 9 (4%) had cribriform architecture. Tertiary Gleason pattern 5 was observed in only one (0.5%) patient. Men with tertiary Gleason pattern 4 had higher median PSA levels (8.4 *versus* 6.6 ng/ml; P=0.01), more frequent extra-prostatic extension (41% *versus* 3%; P<0.001) and positive surgical margins (43% *versus* 10%; P<0.001) than GG1 men without a tertiary pattern. Although GG2- patients had higher percentage Gleason pattern 4 (18% *versus* 3%; P<0.001) and more often tertiary Gleason pattern 5 (9% *versus* 0.5%; P=0.04) than GG1 men with tertiary Gleason pattern 4, PSA levels (9.4 ng/ml *versus* 8.4 ng/ml; P=0.4) and extra-prostatic extension (36% *versus* 41%; P=0.7) were not statistically different.

Clinical outcome

The median follow-up of the entire cohort was 59.6 months (IQR 17.5-113.9). Biochemical recurrence occurred in 112 (18%) men after a median of 29.9 months (IQR 11.6-55.5). GG2- patients had shorter biochemical recurrence-free survival than GG1 patients, while those with cribriform and/or intraductal carcinoma had the worst survival outcome (overall log rank, P<0.001, Figure 4.1). Biochemical recurrence-free survival of men with GG1 disease with tertiary Gleason pattern 4 and GG2- was similar (log rank, P=0.4).

In univariate Cox regression analysis, PSA level (Hazard Ratio (HR) 1.03, 95% Confidence Interval (CI) 1.02-1.04; P<0.001), pT-stage (HR 2.7, 95% CI 1.8-4.1; P<0.001), percentage Gleason pattern 4 (HR 1.03, 95% CI 1.02-1.04; P<0.001), tertiary Gleason pattern 5 (HR 2.4, 95% CI 1.4-4.2; P=0.002), positive surgical margins (HR 3.5, 95% CI 2.4-5.1; P<0.001), positive lymph nodes (HR 20.1, 95% CI 9.8-41.5; P<0.001) and Grade Groups were all significantly associated with biochemical recurrence-free survival (Table 4.2). In multivariable analysis, GG2+ (HR 3.0, 95% CI 1.4-6.3; P=0.004), pT3-stage (HR 1.6, 95% CI 1.0-2.4; P=0.05), positive surgical margins (HR 2.3, 95% CI 1.6-3.5; P<0.001) and positive lymph nodes (HR 7.2, 95% CI 3.0-17.2; P<0.001) had independent predictive value for biochemical recurrence-free survival. Although GG2- patients had shorter biochemical recurrence-free survival (HR 1.9, 95% CI 0.9-3.8) than GG1 patients, this did not meet conventional measures of significance (P=0.08) in multivariate analysis.

During follow-up, 13 (6%) patients with GG2+ developed distant metastasis, of whom 3 had positive lymph nodes at time of radical prostatectomy. While in total 22 (10%) patients with GG2+ had developed either lymph node or distant metastasis, no metastases were identified in any men with GG2- or GG1 prostate cancer at time of operation or during follow-up. Three men deceased from prostate cancer, all having GG2+ disease.

Table 4.1. Clinicopathological characteristics of prostate cancer patients with Grade Group 1 (GG1), Grade Group 2 without cribriform architecture (GG2-) and Grade Group 2 patients with cribriform architecture (GG2+).

	GG1 (n=207)	GG2- (n=192)	P-value*	GG2+ (n=228)
Age (years)	62.5 (63.2; 59.8-66.7)	63.2 (64.0; 59.2-68.1)	0.26	64.2 (64.9; 60.3-67.9)
PSA (ng/ml)	7.0 (6.3; 4.0-9.2)	9.4 (7.7; 5.4-10.5)	<0.001	12.2 (8.3; 6.3-14.0)
pT-stage				
T2	185 (89%)	124 (64%)	<0.001	110 (48%)
T3a	20 (10%)	63 (33%)		90 (40%)
T3b	2 (1%)	5 (3%)		28 (12%)
Gleason pattern 4 (%)	0.6 (0; 0-0)	18 (15; 10-25)	<0.001	24 (20; 15-30)
Invasive cribriform carcinoma	5 (2%)**	0	0.03	204 (90%)
Intraductal carcinoma	4 (2%)**	0	0.05	103 (45%)
Tertiary Gleason pattern 5	1 (0.5%)	18 (9%)	<0.001	31 (14%)
Positive surgical margin status	35 (17%)	52 (27%)	0.014	90 (40%)
Pelvic lymph node dissection	134 (65%)	91 (47%)	<0.001	150 (66%)
Lymph node metastasis	0	0	-	12 (8%)
Biochemical recurrence	16 (8%)	29 (15%)	0.02	67 (29%)
Metastasis	0	0	-	13 (6%)
Disease-specific death	0	0	-	3 (1%)

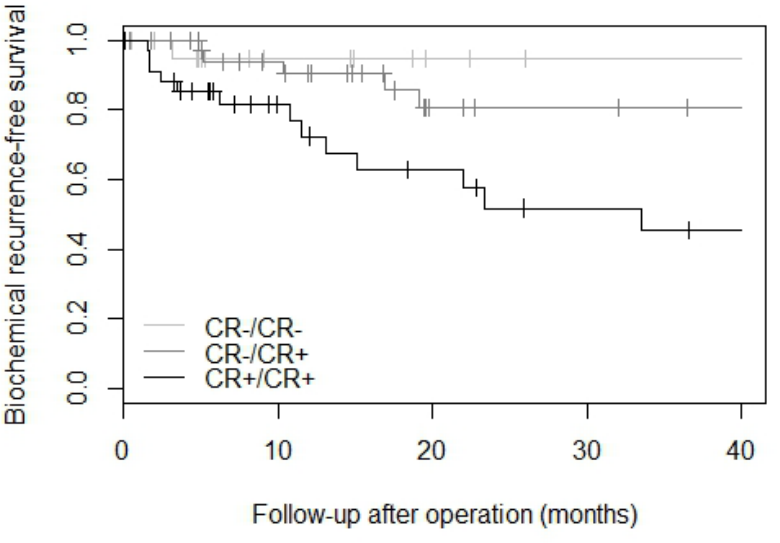
Values represent either mean (median; IQR) or n (%). *P-values represent statistical comparison of GG1 and GG2-. ** Invasive cribriform and intraductal carcinoma as tertiary component in GG1.

Table 4.2. Cox regression analysis of biochemical recurrence-free survival in patients with Grade Group 1 (GG1) and Grade Group 2 prostate cancer patients with (GG2+) and without (GG2-) cribriform architecture.

	Univariate analysis			Multivariable analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	0.99	0.96 - 1.02	0.58	0.99	0.96 - 1.02	0.52
PSA	1.03	1.02 - 1.04	<0.001	1.01	1.00 - 1.02	0.13
pT-stage						
T2	*ref*			*ref*		
T3a	2.7	1.8 - 4.1	<0.001	1.6	1.0 - 2.4	0.05
T3b	9.9	5.8 - 16.9	<0.001	2.7	1.4 - 5.4	0.005
Percentage Gleason 4	1.03	1.02 - 1.04	<0.001	1.0	1.0 - 1.02	0.69
Tertiary Gleason pattern 5	2.4	1.4 - 4.2	0.002	1.4	0.7 - 2.6	0.34
Positive surgical margin status	3.5	2.4 - 5.1	<0.001	2.3	1.6 - 3.5	<0.001
Lymph node metastasis	20.1	9.8 - 41.5	<0.001	7.2	3.0 - 17.2	<0.001
Grade Group						
GG1	*ref*			*ref*		
GG2-	2.7	1.4 - 4.9	0.002	1.9	0.9 - 3.8	0.08
GG2+	6.2	3.6 - 10.7	<0.001	3.0	1.4 - 6.3	0.004

HR = hazard ratio, CI = confidence interval.

Figure 4.1. Kaplan Meier curves for biochemical recurrence-free survival in Grade Group 1 (GG1) and Grade Group 2 prostate cancer patients with (GG2+) and without (GG2-) cribriform architecture. Log rank P<0.001.



Discussion

During the last decade, various studies demonstrated that GG2 prostate cancer patients with invasive cribriform and/or intraductal carcinoma have worse disease outcome than those without.[13, 15, 24, 39, 51, 67] Although it is generally accepted that GG2 prostate cancer patients have more aggressive disease than men with GG1, it is unclear whether this is still the case when those with aggressive cribriform pathology are excluded. In this study, we found that 46% of men with GG2 prostate cancer at radical prostatectomy had neither invasive cribriform nor intraductal carcinoma. These men had significantly higher PSA levels, pT-stage, positive surgical margin rates and shorter biochemical recurrence-free survival than men with GG1 disease. However, none of the 399 men with GG1 or GG2- prostate cancer had metastasis at time of operation or during follow-up, while metastases were identified in 10% of GG2+ patients. These findings indicate that invasive cribriform and/or intraductal carcinoma might have most impact on metastatic disease progression.

Although both invasive cribriform and intraductal carcinoma are pathological features associated with tumour aggressiveness, it is yet unclear how to incorporate these parameters in clinical risk stratification. For instance, Iczkowski et al. proposed to modify current Grade Groups 2 to 4 by reporting the presence of invasive cribriform and intraductal carcinoma denoted with a "C", which would increase the risk groups from 5 to 8.[68] However, it is not yet evident whether clinically relevant differences exist between each of these subgroups or if they partially overlap. Previously, our group found that men with GG2- at biopsy had similar biochemical recurrence rates and disease-specific survival to men with GG1.[32, 39, 52] We currently demonstrate that GG2- is associated with significantly worse clinicopathological characteristics and outcome than GG1 disease. These findings at radical prostatectomy specimens slightly differ from our previous study on biopsy specimens[52]. This might be explained by biopsy sampling artefacts, as upgrading occurs in up to 40% of biopsy GG1 and GG2 prostate cancer.[61, 69]. Furthermore, recent studies indicate that biopsies have a moderate sensitivity of 43 to 47% for detecting cribriform architecture.[59, 66] Despite the moderate concordance of growth patterns between biopsy and radical prostatectomy evaluation, incorporation of cribriform architecture into the Grade Groups has better discriminative value for disease-specific survival and metastasis-free survival.[65] In biopsies, we previously demonstrated that subtraction of one Grade Group, when cribriform architecture is not present, is a simple and valuable modification of the current prostate cancer grading.[65]

Metastasis-free survival of GG2- and GG1 disease was similar in both biopsy and radical prostatectomy studies.[52] GG1 prostate cancer at radical prostatectomy is known to have very low if any risk of metastasis and disease-specific death.[45, 70-77] Since no metastases were identified in

pelvic lymph node dissection or during follow-up of GG2- men, this population seems to have a low risk of metastatic progression as well. This indicates that invasive cribriform and intraductal carcinoma in particular might have impact on the biological potential of developing metastatic disease. In contrast, postoperative biochemical recurrence-free survival is also related to tumour volume parameters and surgical technique, which do not necessarily reflect biological derangement of the disease. Cribriform architecture has been associated with genomic instability and has clonally been related to lymph node metastasis, which might provide a rationale for its aggressive biological behaviour.[18, 78-80]

Forty-two out of 207 (20%) GG1 patients had a tertiary Gleason pattern 4. These patients had worse clinicopathological features than men with pure GG1 disease and were more comparable to GG2- patients. This finding is in line with others reporting on the clinical relevance of tertiary patterns and underlines the importance of reporting them.[81-90]

The strong point of this study is the detailed histological evaluation of the radical prostatectomy specimens, including recently identified clinically relevant pathologic parameters. Limitations of this retrospective investigation are its relatively low number of patients and limited follow-up time of median 59.6 months. Identification of small differences in metastasis-free survival would need a large number of low-risk patients with long-term follow-up.

In conclusion, patients with GG2- prostate cancer at radical prostatectomy have more advanced disease and shorter biochemical recurrence-free survival than men with GG1. However, both groups have very low risk of developing metastatic disease.

Chapter V

Clinicopathological characteristics of glomeruloid architecture in prostate cancer.

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Mod Pathol. 2020 Feb; 33: 1618-1625.

Abstract

Glomeruloid architecture is the least common Gleason 4 growth pattern in prostate adenocarcinoma. Its clinicopathological features and relation with cribriform architecture, which has been recognized as an adverse feature, remains to be established. Our objective was to investigate clinicopathological features of glomeruloid architecture in radical prostatectomies.

We reviewed 1064 radical prostatectomy specimens and recorded Grade Group, pT-stage, margin status, Gleason pattern percentages and growth patterns. Simple and complex glomerulations were distinguished by gland size and intraluminal cribriform protrusions. Clinical endpoint was biochemical recurrence-free survival.

Glomerulations were identified in 365 (34%) specimens. In 472 Grade Group 2 patients, 210 (44%) had simple and 92 (19%) complex glomerulations. Complex glomerulations coincided with cribriform architecture more often than simple glomerulations (67% *versus* 52%; P=0.01). Men with simple glomerulations had significantly lower Prostate Specific Antigen (PSA) levels (9.7 *versus* 12.1 ng/ml; P=0.03), percentage Gleason pattern 4 (19% *versus* 25%; P=0.001), extra-prostatic extension (34% *versus* 50%; P=0.01) and positive surgical margins (25% *versus* 39%; P=0.04) than those with cribriform architecture. Extra-prostatic extension (37%) and positive surgical margins (30%) in men with complex glomerulations resembled those with simple glomeruloid rather than those with cribriform architecture. In multivariate Cox regression analysis adjusted for PSA, pT-stage, margin status and lymph node metastases, cribriform architecture had independent predictive value for biochemical recurrence-free survival (Hazard Ratio (HR) 1.9; 95% Confidence Interval (CI) 1.2-2.9; P=0.004), while simple (HR 0.8; 95% CI 0.5-1.2; P=0.26) and complex (HR 0.9; 95% CI 0.5-1.6; P=0.67) glomerulations did not.

Both simple and complex glomeruloid architecture are associated with better outcome than cribriform architecture in Grade Group 2 prostate cancer patients. Therefore, glomeruloid pattern and particularly complex glomerulations should not be classified as a cribriform growth pattern variant in radical prostatectomy specimens.

Introduction

The Gleason score and Grade Group are the most important parameters for clinical outcome in prostate cancer patients.[8, 12] Gleason pattern 4 is a heterogeneous group of growth patterns including poorly formed, fused, glomeruloid and cribriform structures. The clinical importance of cribriform architecture in prostate cancer has been well-established in recent years, as it is independently associated with disease progression and disease-specific death.[13, 15, 16, 28, 34, 51, 91] Glomeruloid growth pattern consists of dilated malignant glands with intraluminal cribriform protrusions, attached to one side of the gland wall, resembling a renal glomerulus.[12] In 1998, Pacelli et al. first described this growth pattern in relation to tumour grade and stage.[92] Cribriform and glomeruloid growth patterns are often observed together and some have hypothesized that glomeruloid morphology might be a precursor of cribriform architecture.[19, 93] However, more recent studies indicate that glomeruloid pattern is associated with beneficial histopathological features and longer biochemical recurrence-free survival among Gleason score 7 prostate cancer patients.[16, 20]

Interobserver studies have shown that glomeruloid architecture is one of the most reproducible growth patterns in prostate cancer grading.[94, 95] While interobserver agreement is excellent for small glomeruloid protrusions, no consensus exists on the classification of large glomeruloid structures as either glomeruloid or cribriform growth pattern.[95] Since some institutes use cribriform architecture as threshold for active surveillance in Grade Group 2 prostate cancer, distinction between glomeruloid and cribriform growth patterns might have major implications for patient management. Therefore, the aim of this study was to investigate the clinicopathological features and biochemical recurrence-free survival of prostate cancer patients with glomeruloid growth pattern who had undergone radical prostatectomy.

Materials and methods

Patient selection

Patients who had undergone radical prostatectomy for prostate adenocarcinoma in three medical centers in The Netherlands between 2000 and 2017 were included in this study. In total, 854 patients were operated at Erasmus MC, University Medical Center, Rotterdam. In addition, patients from Leiden University Medical Center, Leiden (n=96), and the Netherlands Cancer Institute, Amsterdam (n=137) were selected for high-grade morphology (Grade Group 3-5). We excluded men who had undergone hormonal, radiation or viral therapy (n=23) prior to operation.[40] Radical prostatectomy specimens were fixed in neutral-buffered formalin, after which they were sectioned transversely and completely embedded for diagnostic evaluation. All slides were available for pathology review. This study was approved by the institutional Medical Research Ethics Committee (MEC-2018-1614).

Pathologic evaluation

Radical prostatectomy specimens were reviewed by two investigators (EH, GvL) in common sessions, blinded to clinical outcome. For each specimen the following features were recorded: Gleason score and Grade Group according to the World Health Organization (WHO) and International Society of Urological Pathology (ISUP) 2014 guidelines, pT-stage according to the American Joint Committee on Cancer (AJCC) TNM 8th edition, surgical margin status, presence of individual growth patterns and intraductal carcinoma, and percentage of Gleason pattern 4 and 5.[12, 41] The following Gleason 4 growth patterns were recognized: poorly formed, fused, glomeruloid and cribriform glands.[12, 38] Furthermore, we distinguished two subgroups of glomeruloid growth pattern based on the architecture of intraluminal protrusions (Figure 5.1). Simple glomeruloid architecture was defined as malignant glands with small to medium-sized solid intraluminal cell clusters with unilocular connection to the gland wall. Complex glomeruloid growth pattern had medium to large-sized intraluminal cribriform protrusions with unilocular connection to the gland wall; in some cases the gland wall connection was more extensive. The distinction between glomeruloid and cribriform architecture was arbitrarily made by the extent of gland wall connection, which occupied at least half of the inner glandular surface in cribriform growth and less than half in the glomeruloid pattern. In addition, we distinguished small and large cribriform growth patterns, the latter being defined as cribriform structures with a diameter more than twice the size of adjacent benign glands. Invasive cribriform Gleason pattern 4 was morphologically distinguished from intraductal carcinoma based on the following features: invasive cribriform prostate cancer had an irregular outline, interconnecting fields beyond pre-

existent gland architecture or extension into extra-prostatic tissue. Intraductal carcinoma was morphologically identified if cribriform structures were continuous with pre-existent glands or contained corpora amylacea. If invasive cribriform carcinoma and intraductal carcinoma could not be distinguished by morphological criteria alone, additional basal cell immunohistochemistry was performed. Cribriform glands completely lacking basal cell staining were categorized as invasive cribriform carcinoma. If basal cells were present sporadically, scattered or continuously, the cribriform structures were classified as intraductal carcinoma. Gleason pattern 5 was considered as a tertiary pattern if it occupied less than 5% of the total tumour area.[12, 31, 38] Intraductal carcinoma and tertiary patterns were not incorporated in the Gleason score.

Clinical follow-up

Post-operative clinical follow-up consisted of six-monthly, and later annual monitoring of serum Prostate Specific Antigen (PSA) levels. Biochemical recurrence was defined as PSA levels ≥ 0.2 ng/ml measured at two consecutive points in time, at least three months apart with undetectable PSA levels after operation, or as PSA increase of > 2.0 ng/ml when serum PSA had not declined to zero after the operation. Post-operative lymph node and distant metastases were confirmed by biopsy or multidisciplinary consensus.

Statistical analysis

Continuous variables with normal distribution were analysed using the independent sample Student's t-test. Pearson's chi squared (χ^2) test was used for categorical parameters. Missing PSA values (n=27) were imputed using the median PSA value. Biochemical recurrence-free survival and metastasis-free survival were analysed using Cox proportional hazards regression and visualized by Kaplan-Meier curves. Statistics were performed using SPSS version 24 (IBM, Chicago, IL, USA). Results were considered significant when the two-sided P-value was <0.05.

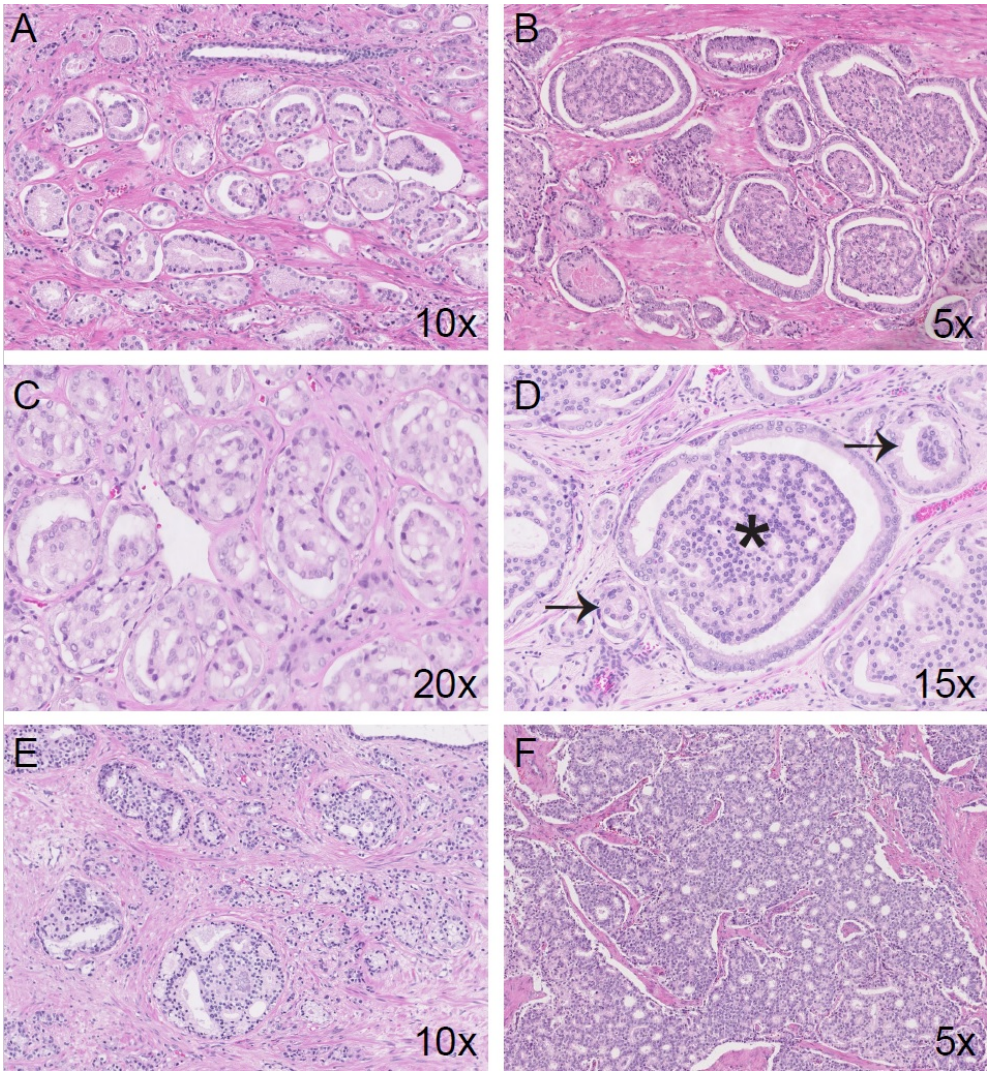


Figure 5.1. Gleason grade 4 cribriform and glomeruloid growth patterns. A. Simple glomeruloid architecture with intraluminal cell clusters, 10x. B. Complex glomeruloid architecture with large cribriform proliferations protruding into the lumen, 5x. C. Simple glomeruloid architecture with vacuolated cytoplasm, therefore not classified as complex glomeruloid architecture, 20x. D. Complex glomeruloid architecture (asterisk) based on a gland wall connection that occupies less than half of the inner gland surface, and simple glomeruloid architecture (arrows) with a small intraluminal protrusion, 15x. E. Small invasive cribriform carcinoma, 10x. F. Large invasive cribriform carcinoma showing expansive confluent fields, 5x.

Results

General patients characteristics

The cohort consisted of 1064 men with a median age of 65 years (interquartile range (IQR) 60-68) and median serum PSA level of 8.3 ng/ml (IQR 6.0-13.2). Median follow-up was 61 months (IQR 20-104). The cohort included 207 (20%) men with Grade Group 1, 472 (44%) with Grade Group 2, 126 (12%) with Grade Group 3, 140 (13%) with Grade Group 4 and 119 (11%) with Grade Group 5 prostate cancer. Pathological stage was distributed as follows: 582 (55%) pT2, 334 (31%) pT3a, 145 (14%) pT3b and 3 (0.3%) pT4 tumours. Surgical margins were positive in 389 (37%) patients. Pelvic lymph node dissection was performed in 664 (62%) patients, of whom 64 (10%) had lymph node metastases.

Gleason 4 growth patterns

Poorly formed and fused glands were observed in 691 (65%) and 613 (58%) men, respectively. Invasive cribriform pattern was present in 519 (49%) men, 189 (18%) of whom had large expansive growth. Intraductal carcinoma was identified in 314 (30%) specimens. In total, 569 (54%) men had invasive cribriform and/or intraductal carcinoma, 190 (33%) of whom had large invasive cribriform growth. Glomeruloid growth was the least frequent Gleason 4 pattern, being present in 365 (34%) men. It was the single Gleason 4 pattern in only 10 (1%) men. Simple glomeruloid glands were present in 352 (33%) men and complex glomeruloid glands in 154 (15%) men. Among patients with glomeruloid architecture, 211 (58%) had simple glomerulations only, 13 (4%) had complex glomerulations only, and 141 (38%) had both simple and complex glomeruloid glands. Simple and complex glomeruloid patterns had concomitant invasive and/or intraductal cribriform carcinoma in 128/211 (61%) and 121/154 (79%) cases ($P<0.001$), respectively. Complex glomeruloid growth did not coincide more often with large compared to small cribriform growth ($P=0.26$).

Simple glomerulations were present in 212 (45%) Grade Group 2, 56 (44%) Grade Group 3, 47 (34%) Grade Group 4 and 28 (24%) Grade Group 5 tumours. In 9 (4%) men with Grade Group 1 tumours, simple glomerulations were present as tertiary Gleason pattern 4. Complex glomerulations were observed in 92 (19%) men with Grade Group 2, 34 (27%) with Grade Group 3, 15 (11%) with Grade Group 4 and 13 (11%) with Grade Group 5 tumours. In none of the cases with Grade Group 1, complex glomerulations were present as tertiary pattern. Simple and complex glomerulations were observed significantly more often in Grade Group 2 and 3 patients compared to Grade Group 4 and 5 patients ($P=0.05$).

Table 5.1. Clinicopathological characteristics of Grade Group 2 prostate cancer patients categorized for cribriform and/or glomeruloid architecture: men with neither glomeruloid nor cribriform architecture (group A), men with simple glomeruloid pattern without complex and/or cribriform architecture (group B), men with complex glomeruloid pattern without invasive and/or intraductal cribriform carcinoma (group C), and men with invasive and/or intraductal cribriform carcinoma regardless of presence of glomerulations (group D).

	Group A n=130	*Group B n=60*	*Group C n=30*	*Group D n=252*
Age	63.3 (64.1; 59.1-68.3)	63.4 (64.6; 59.0-69.1)	63.4 (63.6; 60.1-67.2)	64.2 (64.7; 60.3-67.9)
PSA	9.1 (7.6; 5.0-11.1)	9.7 (8.2; 6.2-11.0)	13.1 (9.3; 6.5-13.7)	12.1 (8.3; 6.3-13.5)
pT-stage				
T2	83 (64%)	40 (66%)	19 (64%)	126 (50%)
T3a	43 (33%)	19 (32%)	10 (33%)	97 (38%)
T3b	4 (3%)	1 (2%)	1 (3%)	29 (12%)
Gleason pattern 4 (%)	18 (15; 10-26)	19 (15; 10-30)	20 (20; 10-30)	25 (25; 20-34)
Cribriform	0	0	0	223 (89%)
Fused	81 (62%)	42 (70%)	22 (73%)	180 (71%)
Ill-defined	103 (79%)	44 (73%)	19 (63%)	198 (79%)
Glomeruloid	0	60 (100%)	30 (100%)	126 (50%)
simple	0	60 (100%)	28 (93%)	124 (49%)
complex	0	0	30 (100%)	62 (25%)
Intraductal carcinoma	0	0	0	113 (45%)
Tertiary Gleason 5	13 (10%)	6 (10%)	3 (10%)	35 (14%)
PSM	35 (27%)	15 (25%)	9 (30%)	97 (39%)
PLND	64 (49%)	22 (37%)	16 (53%)	160 (64%)
Lymph node metastasis	0	0	0	13 (5%)
Biochemical recurrence	22 (17%)	8 (13%)	4 (13%)	73 (29%)
Metastasis	0	0	0	17 (7%)
Disease-specific death	0	0	0	3 (1%)

*Values denote either mean (median; IQR) or n (%). PSM = positive surgical margin.

Glomeruloid architecture in Grade Group 2 prostate cancer

Since clinical impact of glomeruloid growth pattern classification is most relevant for Grade Group 2 prostate cancer patients, we performed further analyses in this subpopulation of 472 men. Of these, 216 (46%) had glomerulations: 212 (45%) had simple and 92 (20%) complex glomeruloid structures. Simple glomerulations only were present in 124 (57%) men, complex glomerulations only in 4 (2%) men, and both patterns occurred concurrently in 88 (41%) cases. Invasive and/or intraductal cribriform carcinoma was present in 252 (53%) men with Grade Group 2 prostate cancer, 34 (13%) of whom had large invasive cribriform carcinoma. Glomeruloid architecture was associated with presence of invasive and/or intraductal cribriform carcinoma, as 126/216 (58%) men with glomerulations had coexistent cribriform architecture compared to 126/256 (49%) men without glomerulations (P=0.05). Complex glomeruloid structures (62/92, 67%) were more frequently concomitant with invasive and/or intraductal cribriform carcinoma than simple glomerulations (64/124, 52%, P=0.01).

Further analyses on glomeruloid and cribriform growth patterns were performed in four subgroups (Table 5.1), to investigate the relation between glomeruloid and cribriform architecture. These subgroups consisted of men with neither glomeruloid nor cribriform architecture, thus with poorly formed and fused glands only (n=130, group A), men with simple glomeruloid pattern without complex and/or cribriform architecture (n=60, group B), men with complex glomeruloid pattern without invasive and/or intraductal cribriform carcinoma (n=30, group C), and men with invasive and/or intraductal cribriform carcinoma regardless of presence of glomerulations (n=252, group D). Patients with invasive cribriform and/or intraductal carcinoma (group D) had significantly higher percentage Gleason pattern 4 (25% *versus* 18%; P<0.001), pT-stage (50% *versus* 36% pT3; P=0.003) and positive surgical margin rates (39% *versus* 27%; P=0.02) than those with poorly formed and fused glands only (group A). Men with simple glomerulations (group B) had similar PSA levels (9.7 *versus* 9.1 ng/ml; P=0.6), percentage Gleason pattern 4 (19% *versus* 18%; P=0.6), pT-stage (34% *versus* 36% pT3; P=0.8), and positive surgical margins (25% *versus* 27%; P=0.7) to men with poorly formed and fused glands only (group A). Compared to men with invasive cribriform and/or intraductal carcinoma (group D), men with simple glomerulations (group B) had significantly lower PSA levels (9.7 *versus* 12.1 ng/ml; P=0.03), percentage Gleason pattern 4 (19% *versus* 25%; P=0.001), pT-stage (34% *versus* 50% pT3; P=0.01), and positive surgical margins (25% *versus* 39%; P=0.04). Although men with complex glomeruloid glands only (group C) had higher PSA levels (13.1 *versus* 9.7 ng/ml; P=0.05) than men with simple glomeruloid growth pattern (group B), percentage Gleason pattern 4 (20% *versus* 19%; P=0.6), presence of tertiary Gleason pattern 5 (both 10%; P=1.0), pT-stage (37% *versus* 34% pT3; P=0.8) and surgical margin status (30% *versus* 25%; P=0.6) were similar. Patients with complex glomeruloid glands (group C)

had similar PSA levels (13.1 *versus* 12.1 ng/ml; P=0.7) to those with invasive cribriform and/or intraductal carcinoma (group D), but its percentage Gleason pattern 4 was significantly lower (20% *versus* 25%; P=0.04). Although the complex glomeruloid sample size (n=30) was too low for reliable statistical analysis, percentage of Gleason pattern 4, presence of tertiary pattern 5, pT-stage and positive surgical margin status resembled simple glomerulations (group B) rather than cribriform architecture (group D). Among patients with invasive and/or intraductal cribriform carcinoma (group D), no significant differences were found in PSA, pT-stage, percentage Gleason pattern 4, presence of tertiary Gleason pattern 5 and surgical margin status between those with and without glomeruloid architecture (*data not shown*).

Clinical outcome of Grade Group 2 patients

Median follow-up of Grade Group 2 patients was 57 months (IQR 14-99). Biochemical recurrence occurred in 22/130 (17%) men with poorly formed and fused glands only (group A), in 8/60 (13%) men with simple glomerulations (group B), in 4/30 (13%) men with complex glomerulations (group C) and in 73/252 (29%) men with cribriform architecture (group D). Survival curves are shown in Figure 5.2. No statistically significant difference in biochemical recurrence-free survival was found between men with poorly formed and fused glands only (group A), simple glomeruloid glands (group B) or complex glomeruloid glands (group C; log rank P=0.38). Patients with invasive and/or intraductal cribriform architecture (group D) had significantly shorter biochemical recurrence-free survival (log rank P<0.001) than patients with simple (group B, P=0.006) and complex glomeruloid glands (group C, P=0.05).

Cox regression analysis showed that intraductal carcinoma (Hazard ratio (HR) 2.7, 95% CI 1.8-4.0, P<0.001), small invasive cribriform carcinoma (HR 1.9, 95% CI 1.3-3.0, P=0.002) and large invasive cribriform carcinoma (HR 6.3, 95% CI 3.6-11.1, P<0.001) were associated with shorter biochemical recurrence-free survival in univariate analysis (Table 5.2). Adjusted for PSA level, pT-stage, surgical margin and pelvic lymph node metastases, large invasive cribriform carcinoma remained an independent predictor for biochemical recurrence (HR 3.8, 95% CI 2.1-6.8, P<0.001) in multivariable analysis. Simple (HR 0.8; 95% CI 0.6-1.4; P=0.64) and complex (HR 0.6; 95% CI 0.4-1.2; P=0.19) glomeruloid growth patterns were not associated with biochemical recurrence-free survival in univariate or multivariable analysis. Metastases (n=17) and disease-specific death (n=3) only occurred in patients with presence of invasive cribriform and/or intraductal carcinoma, and not in other subgroups.

Figure 5.2. Kaplan-Meier curves of biochemical recurrence-free survival in Grade Group 2 patients with cribriform and/or glomeruloid architecture, stratified for subgroups A to D.

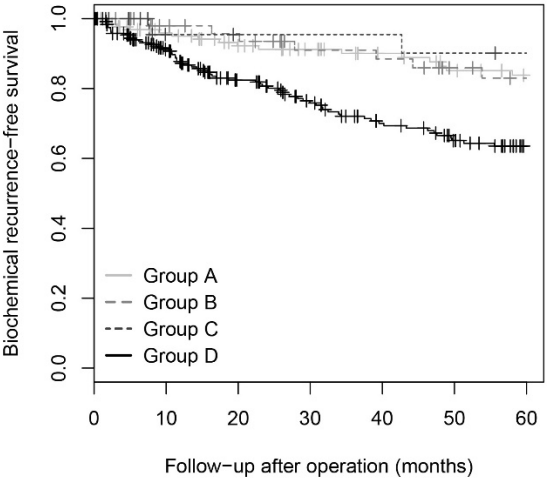


Table 5.2. Cox regression analysis for biochemical recurrence-free survival in Grade Group 2 prostate cancer patients.

	Univariate			Multivariable		
	HR	95% CI	P-value	HR	95% CI	P-value
PSA	1.03	1.01-1.04	<0.001	1.01	1.00-1.02	0.06
pT-stage						
T2	*ref*			*ref*		
T3a	1.8	1.2-2.7	0.008	1.5	1.0-2.3	0.07
T3b	4.9	2.8-8.5	<0.001	1.9	1.0-3.7	0.07
Positive surgical margins	2.5	1.7-3.7	<0.001	2.4	1.6-3.7	<0.001
Pelvic lymph node metastases	15.2	7.6-30.3	<0.001	7.3	3.2-16.8	<0.001
Intraductal carcinoma	2.7	1.8-4.0	<0.001	1.4	0.9-2.3	0.15
Invasive cribriform carcinoma						
Small cribriform	1.9	1.3-3.0	0.002	1.2	0.7-1.8	0.48
Large cribriform	6.3	3.6-11.1	<0.001	3.8	2.1-6.8	<0.001
Glomeruloid growth pattern						
Simple	0.8	0.5-1.2	0.20	0.8	0.6-1.4	0.64
Complex	1.0	0.6-1.6	0.86	0.6	0.4-1.2	0.19

*HR = hazard ratio, CI = confidence interval.

Discussion

In this study we investigated the clinicopathological features of glomeruloid Gleason pattern 4 architecture. Overall glomeruloid architecture was present in 34% of radical prostatectomy specimens. In Grade Group 2 patients, simple glomeruloid was seen in 45% and complex glomeruloid growth in 20% of men. Men with simple glomeruloid glands only had similar clinicopathological characteristics to those with poorly formed and fused glands. Although patients with complex glomeruloid glands had higher PSA levels than men with poorly formed, fused or simple glomeruloid glands, no significant difference was observed for pT-stage, percentage Gleason pattern 4, or biochemical recurrence-free survival. Biochemical recurrence and metastasis occurred significantly more often in patients with invasive cribriform and/or intraductal carcinoma. Despite their morphological resemblance, men with complex glomerulations had better outcome than those with cribriform architecture. Therefore, complex glomeruloid pattern should not be classified as a variant of the more aggressive cribriform growth pattern in radical prostatectomy specimens.

Pathological grading of the glomeruloid growth pattern has been uncertain for a long time. At the 2005 ISUP conference, no consensus was reached on grading glomeruloid pattern due to lack of scientific evidence for its prognostic value.[11] In 2009, Lotan and Epstein studied 45 prostate cancer biopsies with glomeruloid features and found they were surrounded by Gleason pattern 4 structures in 80% and Gleason pattern 5 in 4% of cases.[19] The authors noted that half of the glomeruloid structures were accompanied by cribriform pattern, which is in concordance with our findings. For this reason it was unanimously consented at the 2014 ISUP conference that glomeruloid glands should be assigned Gleason pattern 4, regardless of morphology.[12]

Since the increased awareness of the dismal outcome of cribriform Gleason pattern 4, glomeruloid growth pattern has also been included in clinicopathological studies. Among 350 radical prostatectomies with Gleason score 7 prostate cancer, Choy et al. found that patients with glomeruloid pattern had improved five-year biochemical recurrence-free survival in multivariable analysis, but had shorter survival than those with Gleason score 6.[20] Glomeruloid growth pattern was associated with improved, although not statistically significant, metastasis-free survival of Gleason score 7 men in our previous radical prostatectomy study.[16] However, both studies did not focus on glomeruloid architecture specifically and were performed on a Gleason score 7 cohort. The study of Kweldam et al. had a case-control study design and used metastasis-free survival as endpoint. We investigated glomeruloid architecture in a larger cohort of Grade Group 1 to 5 prostate cancer and distinguished simple and complex glomerulations, which was not done in previous manuscripts. In the current study, clinical outcome of patients with glomeruloid

architecture was better than of those with cribriform architecture, and did not differ from those with poorly formed and fused glands only. Statistical analyses were hampered by the fact that glomeruloid architecture is rarely observed as single Gleason pattern 4, mostly coexisting with other growth patterns.

Glomeruloid architecture is not a homogeneous Gleason pattern 4 subgroup. As previously reported by Lotan and Epstein, most glomerulations consisted of relatively small dilated glands, but some contain larger glomeruloid protrusions.[19] Furthermore, a subset of glomerulations has fibrovascular cores. This is in line with three-dimensional renderings of glomeruloid structures which revealed an interconnecting network of tubules resembling Gleason pattern 3 glands with nodular epithelial proliferations near tubular branching points, or markedly curved tubules with small fibrovascular cores.[96] While glomeruloid architecture is the most reproducible Gleason 4 growth pattern, interobserver variability exists for classifying glomeruloid structures with larger intraluminal cribriform protrusions.[95] In this case, classification as either glomeruloid or cribriform architecture is uncertain, because the extent of cribriform intraluminal gland wall attachment has not been defined. Among Grade Group 2 prostate cancer patients, two-thirds of specimens with complex glomeruloid pattern had concomitant cribriform pattern which might suggest that both patterns are related. Complex cribriform pattern for instance could be a tangential sectioning of cribriform architecture expanding into malignant tubules or might represent a precursor lesion. While designation of these structures as either cribriform or glomeruloid does not have clinical relevance if they coexist with cribriform architecture, their distinction might be important in diagnostic biopsies without cribriform structures. Active surveillance is mostly offered to men with biopsy Grade Group 1 disease, but some surveillance protocols are also including men with Grade Group 2 prostate cancer without cribriform architecture and/or low Gleason pattern 4 percentages.[45, 61-64, 97] Therefore, classifying complex glomeruloid structures might directly affect clinical decision-making. Our current findings in radical prostatectomy specimens indicate that men with complex glomeruloid structures without concomitant cribriform growth have better outcome than those with cribriform architecture. Therefore, at this moment insufficient evidence exists for classifying complex glomeruloid pattern as cribriform Gleason pattern 4.

The strong point of this study was the detailed histological review of a large cohort of radical prostatectomy specimens. In this study the distinction between glomeruloid and cribriform architecture was arbitrarily made by the extent of gland wall connection, which occupied at least half of the inner glandular surface in cribriform growth and less in the glomeruloid pattern. No standard definition of complex glomeruloid pattern has been formulated yet. Therefore, it is important that future studies define the morphological criteria for distinguishing complex

glomeruloid and cribriform growth patterns. Some pathologists might have interpreted the complex glomeruloid cases as cribriform, however our current results do not provide evidence for this classification. Finally, this study was limited by its retrospective design and relatively short follow-up of 57 months.

In conclusion, glomeruloid architecture is observed in 34% of radical prostatectomy specimens. Patients with simple glomeruloid glands have similar clinicopathological characteristics and biochemical recurrence-free survival as poorly formed and fused glands in Grade Group 2 prostate cancer. Although complex glomeruloid glands are more often coexistent with cribriform growth pattern, biochemical recurrence and metastasis occurred significantly less frequently in this subgroup. Therefore, complex glomeruloid architecture does not classify as a cribriform Gleason 4 growth pattern in radical prostatectomy specimens. Further studies are needed to address the significance of glomeruloid architecture in prostate biopsies.

Chapter VI

Cribriform architecture in radical prostatectomies predicts oncological outcome in Grade Group 4 prostate cancer patients.

Hollemans E, Verhoef EI, Bangma CH, Rietbergen J, Osanto S, Pelger RCM, van Wezel T, van der Poel H, Bekers E, Helleman J, Roobol MJ, van Leenders GJLH.

Mod Pathol. 2021 Jan; 34: 184-193.

Abstract

The Gleason score is an important parameter for clinical outcome in prostate cancer patients. Gleason score 8 is a heterogeneous disease including Gleason score 3+5, 4+4, and 5+3 tumours, and encompasses a broad range of tumour growth patterns. Our objective was to characterize individual growth patterns and identify prognostic parameters in Gleason score 8 prostate cancer patients.

We reviewed 1,064 radical prostatectomy specimens, recorded individual Gleason 4 and 5 growth patterns as well as presence of intraductal carcinoma, and evaluated biochemical recurrence- and metastasis-free survival.

Gleason score 8 disease was identified in 140 (13%) patients, of whom 76 (54%) had Gleason score 3+5, 46 (33%) 4+4, and 18 (13%) 5+3 disease. Invasive cribriform and/or intraductal carcinoma (n=87, 62%) was observed more frequently in Gleason score 4+4 (93%) than 3+5 (47%; P<0.001) and 5+3 (44%; P<0.001) patients. Gleason pattern 5 was present in 110 (79%) men: as single cells and/or cords in 99 (90%) and solid fields in 32 (29%) cases. Solid field pattern 5 coexisted with cribriform architecture (23/32, 72%) more frequently than non-solid pattern 5 cases (36/78, 46%, P=0.02). In multivariable analysis including age, Prostate-Specific Antigen, pT-stage, surgical margin status and lymph node metastases, presence of cribriform architecture was an independent parameter for biochemical recurrence-free (Hazard Ratio (HR) 2.0, 95% Confidence Interval (CI) 1.0-3.7; P=0.04) and metastasis-free (HR 3.5, 95% CI 1.0-12.3; P=0.05) survival.

In conclusion, invasive cribriform and/or intraductal carcinoma occurs more frequently in Gleason score 4+4 prostate cancer patients than in Gleason score 3+5 and 5+3, and is an independent parameter for biochemical recurrence and metastasis. Therefore, cribriform architecture has added value in risk stratification of Gleason score 8 prostate cancer patients.

Introduction

The Gleason grading system for prostate cancer is based on classification of histomorphological growth patterns.[8] At the 2014 meeting of the International Society of Urological Pathology (ISUP), consent was reached that a Grade Group should be reported in conjunction with the Gleason score, based on the initial work of Pierorazio et al., which was endorsed by the World Health Organization (WHO) in 2016.[12, 38, 98]. The Grade Group system is comprehensive and facilitates patient communication as it labels Gleason score 2-6 as Grade Group 1 and emphasizes the important distinction between Gleason score 3+4=7 (Grade Group 2) and 4+3=7 (Grade Group 3) prostate cancer. Grade Group 4 prostate cancer encompasses Gleason score 8 tumours, including Gleason score 3+5, 5+3, and 4+4.[58, 99] However, it is not yet clear whether these three Gleason score 8 subgroups have similar clinical outcome.

The importance of distinguishing individual prostate cancer growth patterns is increasingly being acknowledged. Gleason pattern 4 encompasses four major growth patterns, including poorly formed, fused, glomeruloid and cribriform glands.[12] The clinical relevance of cribriform architecture in prostate cancer has been well established in recent years, as it is associated with biochemical recurrence, metastasis and disease-specific death.[13, 15, 16, 28, 34, 51, 91] Intraductal carcinoma is characterized by a proliferation of malignant epithelial cells with cribriform or solid architecture distending pre-existent acini and prostatic ducts with preservation of basal cells.[12] Although not incorporated in the Gleason score or Grade Group, intraductal carcinoma is independently associated with adverse oncological outcome.[28, 35, 39] The adverse impact of invasive cribriform and intraductal carcinoma has mainly been studied in Gleason score 3+4 prostate cancer, as it might affect clinical decision-making in this patient population in particular. Some studies indicate that presence of invasive cribriform and intraductal carcinoma also has independent predictive value in Gleason score 8 prostate cancer patients.[39, 100]

While the impact of cribriform architecture is well recognized, little is known about the clinical relevance of individual Gleason 5 growth patterns.[13] Gleason pattern 5 encompasses tumour growth in single cells, cords and solid fields.[12] Furthermore, presence of comedonecrosis is considered Gleason pattern 5, whether it is present within papillary, cribriform or solid fields. Of notice, recent studies have shown that comedonecrosis more commonly occurs in intraductal carcinoma than in invasive carcinoma, requiring basal cell immunohistochemistry for their distinction.[101-103] While Gleason score 8 prostate cancer is generally considered a high-risk disease requiring immediate therapeutic intervention, analysis of individual Gleason 4 and 5 growth patterns might attribute to risk stratification and optimize personalized treatment decisions. The objective of this study is to compare the clinical characteristics and outcome of Gleason score 3+5,

5+3, and 4+4 subgroups and to investigate the impact of invasive cribriform and/or intraductal carcinoma in Gleason score 8 radical prostatectomy specimens.

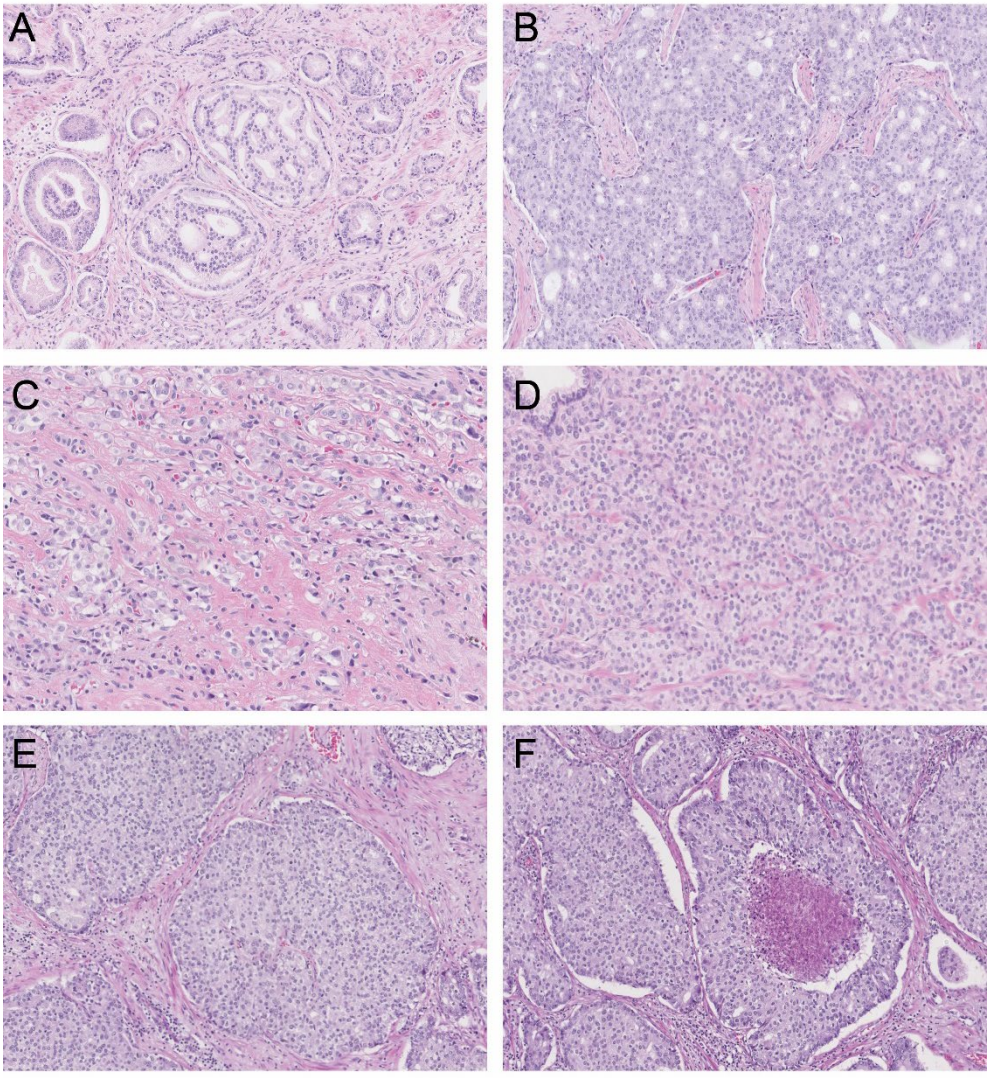


Figure 6.1. Gleason pattern 4 and pattern 5 tumor morphology. A. Gleason pattern 4, small invasive cribriform structures, 15x. B. Gleason pattern 4, large invasive cribriform structures, 10x. C. Gleason pattern 5, cords, 20x. D. Gleason pattern 5, small solid nests with subtle intervening stroma, 20x. E. Gleason pattern 5, medium to large sized solid fields, 15x. F. Gleason pattern 5, comedonecrosis in a solid field, 15x.

Materials and methods

Patient selection

Patients who had undergone radical prostatectomy for prostatic adenocarcinoma from three university medical centers in The Netherlands between 2000 and 2017 were included in this study; 854 patients were operated at Erasmus MC, University Medical Center, Rotterdam; 96 at Leiden University Medical Center (LUMC), Leiden; and 137 at Antoni van Leeuwenhoek Hospital, the Netherlands Cancer Institute (NKI), Amsterdam. Whereas the radical prostatectomies from Erasmus MC were consecutive, those from LUMC and NKI were selected for presence of Gleason score 4+3 to 10 in the original pathology report. We excluded men who had undergone hormonal, radiation and/or viral therapy (n=23) prior to operation.[40] Radical prostatectomy specimens were fixed in neutral-buffered formalin, after which they were sectioned transversely and embedded entirely for diagnostic purposes. All slides were available for pathology review. This study was approved by the institutional Medical Research Ethics Committee (MEC-2018-1614).

Pathologic evaluation

All 1,064 radical prostatectomy specimens were reviewed in common sessions by two investigators (EH, GvL), blinded to clinical outcome. For each specimen the following features were recorded: Gleason score and Grade Group according to the 2014 ISUP/2016 WHO guidelines, pT-stage according to the American Joint Committee on Cancer (AJCC) TNM 8th edition, surgical margin status, presence of intraductal carcinoma, and percent Gleason 4 and 5 growth patterns.[12, 41] In case of multifocality we only monitored the characteristics of the index tumour defined as the tumour with the highest grade, stage or volume.

The following Gleason 4 growth patterns were recognized: poorly formed, fused, glomeruloid and cribriform glands.[12, 38] Furthermore, we distinguished small and large cribriform gland architecture (Figure 6.1, A-B), since the latter is associated with more aggressive behaviour.[51] Large cribriform structures were defined as having a diameter more than twice the size of adjacent benign glands. We examined the following Gleason 5 growth patterns: single cells, cords, and solid fields (Figure 6.1, C-F). Single cells and cords were grouped for analysis. Solid fields were divided into those with small solid nests containing 10 to 30 cells, and those consisting of medium to large solid fields with more than 30 cells. In case comedonecrosis was present in invasive cribriform or solid fields, this was considered Gleason pattern 5. Invasive cribriform Gleason pattern 4 and solid pattern 5 either with or without comedonecrosis were morphologically distinguished from intraductal carcinoma based on the following features: invasive cribriform and solid prostate cancer had irregular borders or formed interconnecting fields, well exceeding the

outline of distended pre-existent glands, or extended into periprostatic adipose tissue, ejaculatory ducts or seminal vesicles. Intraductal carcinoma was continuous with pre-existent glands lined by basal cells, or contained corpora amylacea. In case invasive cribriform or solid carcinoma and intraductal carcinoma could not be differentiated by morphological criteria alone, additional basal cell immunohistochemistry was performed. Basal cell immunohistochemistry (34BE12) was performed in 189/854 (22%) radical prostatectomy specimens from Erasmus MC, including 14/31 (45%) Gleason score 8 tumours with cribriform or solid architecture; no paraffin blocks were available from the other hospitals. If basal cells were completely absent, the lesion was classified as either invasive cribriform Gleason pattern 4 or solid pattern 5 carcinoma. When sporadic, scattered or continuous basal cells were identified, the lesion was considered intraductal carcinoma. Intraductal carcinoma and tertiary patterns were not incorporated in the Gleason score.[12, 31, 38] Minor high-grade components occupying <5% of the tumour volume were considered as tertiary pattern. The Grade Group concordance rate at revision was 88/135 (65%) for radical prostatectomies from NKI and 39/94 (41%) for specimens from LUMC.

Clinical follow-up

Clinical follow-up after radical prostatectomy consisted of six-monthly, and later annual monitoring of serum Prostate-Specific Antigen (PSA) levels. Biochemical recurrence was defined as PSA levels ≥ 0.2 ng/ml measured at two consecutive points in time, at least three months apart with undetectable PSA levels after operation, or as PSA increase of > 2.0 ng/ml when serum PSA had not declined to zero after operation. Post-operative lymph node and distant metastases were confirmed by biopsy or multidisciplinary consensus.

Statistical analysis

Continuous variables with normal distribution were analysed using the independent sample Student's t-test for two groups, or One-way ANOVA for ≥3 groups. Variables without normal distribution were analysed using the Mann-Whitney U test for two groups, or Kruskal-Wallis test for ≥3 groups. For comparison of categorical parameters Pearson's chi squared (χ^2) test was used, and Fisher's exact test in case of small numbers (n≤20). Missing PSA values (n=27) were imputed using the median PSA value. Biochemical recurrence-free survival and metastasis-free survival were analysed using Cox proportional hazards model and visualized by Kaplan-Meier curves. Statistics were performed using SPSS version 24 (IBM, Chicago, IL, USA). Results were considered significant when the two-sided P-value was <0.05.

Results

Characteristics of Gleason score 8 prostate cancer patients

Out of 1,064 radical prostatectomy specimens, 140 (13%) had Gleason score 8 prostate cancer. The median age of Gleason score 8 patients was 65.3 years (interquartile range (IQR) 61.4-68.5 years) and median serum PSA level was 10.0 ng/ml (IQR 7.2-16.0 ng/ml). Gleason scores were distributed as follows: 76 (54%) men had Gleason score 3+5, 46 (33%) Gleason score 4+4, and 18 (13%) Gleason score 5+3. Pathologic tumour stage was T2 in 67 (48%) men, T3a in 44 (31%) and T3b in 28 (20%). One (1%) patient had a T4 tumour and was grouped with T3b tumours for further analysis. Positive surgical margins were present in 68 (49%) cases. Pelvic lymph node dissection was performed in 91 (65%) men, 12 (9%) of whom had lymph node metastasis. Median follow-up time was 68.7 months (IQR 36.7-102.8).

Clinicopathological features and outcome of Gleason score 3+5, 4+4 and 5+3

The clinicopathological features of Gleason score 8 patients stratified for Gleason score are shown in Table 6.1. The median PSA level of patients with Gleason score 5+3 prostate cancer was 13.4 ng/ml (IQR 8.8-26.8 ng/ml), significantly higher than for men with Gleason score 3+5 (10.0 ng/ml; IQR 7.4-15.0 ng/ml; P=0.05) and Gleason score 4+4 (8.9 ng/ml; IQR 6.9-16.0 ng/ml; P=0.03). PSA levels of Gleason score 3+5 and 4+4 were comparable (P=0.45). Age, pT-stage, surgical margin status and lymph node metastases were not significantly different between groups. While Gleason pattern 4 constituted ≥95% of the tumour volume in Gleason score 4+4 by definition, it was present in 73/76 (96%) Gleason score 3+5 and 12/18 (67%) 5+3 tumours. The median percentage of Gleason pattern 4 was 30% (IQR 20%-35%) in 3+5 tumours and 18% (IQR 0%-21%) in 5+3 tumours (P<0.001). Tertiary (<5%) Gleason pattern 5 was observed in 16/46 (35%) Gleason score 4+4 tumours.

Biochemical recurrence and post-operative distant metastasis were observed in 68 (49%) and 36 (26%) patients, respectively. Twenty-nine (63%) men with Gleason score 4+4 tumours experienced biochemical recurrence compared to 31 (41%, P=0.02) with Gleason score 3+5 and 8 (44%, P=0.78) with 5+3. Biochemical recurrence-free survival was significantly shorter for patients with Gleason score 4+4 than Gleason score 3+5 (log rank P=0.02) prostate cancer. Gleason score 5+3 had the lowest absolute number of events and did not significantly differ from Gleason score 3+5 (log rank P=0.82) and Gleason score 4+4 (log rank P=0.26, Figure 6.2). A similar trend was found for post-operative metastasis. Metastases occurred in 18 (39%) men with Gleason score 4+4 compared to 14 (18%, P=0.01) with Gleason score 3+5 and 4 (22%, P=0.20) men with Gleason score 5+3. Metastasis-free survival was significantly shorter

Table 6.1. Gleason score 8 patients stratified for individual Gleason score (GS).

	All n=140	*GS 3+5 (n=76)*	*GS 4+4 (n=46)*	*GS 5+3 (n=18)*	*P-value*
Age (years)	64.7 (65.3; 61.4-68.5)	64.3 (64.5; 60.7-68.2)	65.6 (66.2; 62.4-69.2)	64.5 (65.2; 60.4-67.2)	0.46
PSA (ng/ml)	12.9 (10.0; 7.2-16.0)	12.5 (10.0; 7.4-15.0)	11.5 (8.9; 6.9-16.0)	18.5 (13.4; 8.8-26.8)	0.07
pT-stage	67 (48%)	41 (54%)	19 (41%)	7 (39%)	0.35
T2	44 (31%)	19 (25%)	19 (41%)	6 (33%)	
T3a	29 (21%)	16 (21%)	8 (18%)	5 (28%)	
T3b/T4					
Overall cribriform	87 (62%)	36 (47%)	43 (93%)	8 (44%)	<0.001
Gleason pattern 4	*83 (59%)*	*33 (43%)*	*42 (91%)*	*8 (44%)*	<0.001
Small cribriform	*38 (27%)*	*6 (8%)*	*28 (61%)*	*4 (22%)*	<0.001
Large cribriform					
Intraductal carcinoma	48 (34%)	21 (28%)	23 (50%)	4 (22%)	0.02
Gleason pattern 5	99 (71%)	72 (95%)	10 (22%)	17 (94%)	<0.001
Single cells and/or cords	23 (16%)	16 (21%)	1 (2%)	6 (33%)	0.003
Small solid nests	15 (11%)	4 (5%)	5 (11%)	6 (33%)	0.006
Medium to large solid fields	9 (6%)	2 (3%)	7 (15%)	0	0.02
Comedonecrosis					
Positive surgical margins	68 (49%)	40 (53%)	20 (44%)	8 (44%)	0.58
Pelvic lymph node dissection	91 (65%)	47 (62%)	29 (63%)	15 (83%)	0.22
Lymph node metastasis	12 (13%)	5 (11%)	4 (14%)	3 (20%)	0.54
Biochemical recurrence	68 (49%)	31 (41%)	29 (63%)	8 (44%)	0.05
Metastasis	36 (26%)	14 (18%)	18 (39%)	4 (22%)	0.04
Disease-specific death	12 (9%)	4 (5%)	7 (15%)	1 (6%)	0.17

Values denote either mean (median; IQR) or n (%). PSA = Prostate Specific Antigen.

Figure 6.2. Kaplan-Meier curves of A. biochemical recurrence-free survival (log rank P=0.001), B. metastasis-free survival (log rank P<0.001) and C. disease-specific survival (log rank P=0.01) in Gleason score 8 patients stratified for Gleason score.

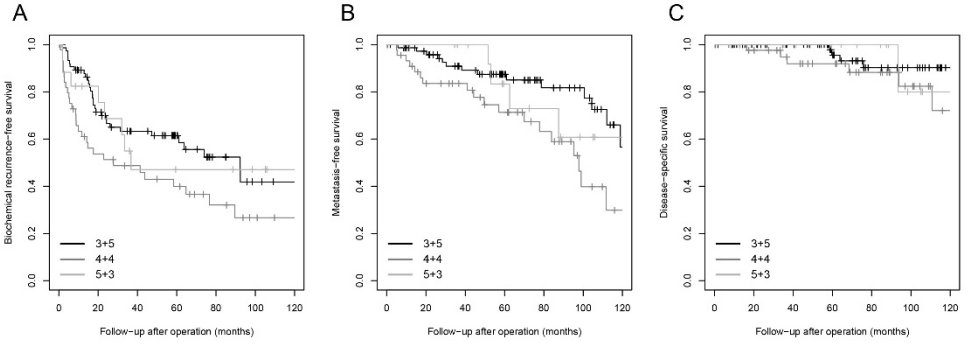
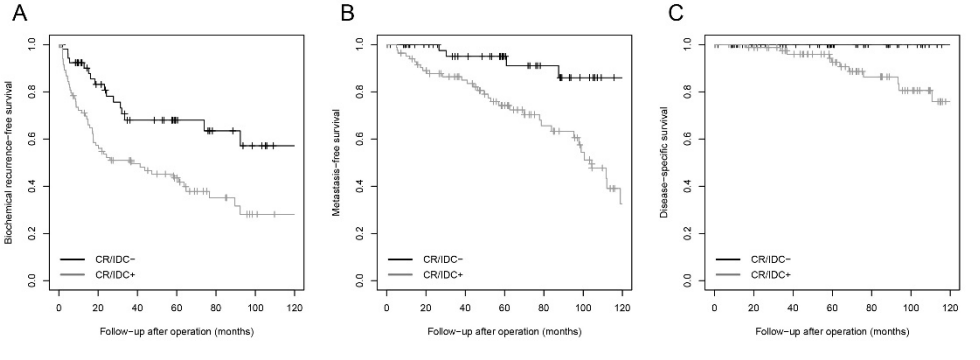


Figure 6.3. Kaplan-Meier curves of A. biochemical recurrence-free survival (log rank P=0.001), B. metastasis-free survival (log rank P<0.001) and C. disease-specific survival (log rank P=0.01) in Gleason score 8 patients with invasive and/or intraductal cribriform carcinoma (CR/IDC+) and without invasive and/or intraductal cribriform carcinoma (CR/IDC-).



for patients with Gleason score 4+4 than Gleason score 3+5 (log rank P=0.006) prostate cancer. Gleason score 5+3 did not significantly differ from Gleason score 3+5 (log rank P=0.63) and Gleason score 4+4 (log rank P=0.25). The number of disease-specific deaths (n=12, 9%) was too low for subgroup analysis.

Cribriform architecture in Gleason score 8 prostate cancer

Invasive and/or intraductal cribriform carcinoma was present in 87 (62%) men, of whom 36 (41%) had Gleason score 3+5, 43 (49%) Gleason score 4+4, and 8 (10%) Gleason score 5+3 tumours. Of these, 83 (95%) had invasive and 48 (55%) had intraductal cribriform carcinoma. Both patterns were concurrently present in 44 (51%) men. Invasive cribriform carcinoma only was seen in 39 (44%) men and intraductal cribriform carcinoma only in 4 (5%) men. Large cribriform carcinoma was present in 37 (43%) men with cribriform architecture and was always accompanied by small cribriform carcinoma. Invasive and/or intraductal cribriform carcinoma was observed more frequently in Gleason score 4+4 than in Gleason score 3+5 (93% *versus* 47%, P<0.001) and 5+3 (93% *versus* 44%, P<0.001) tumours. Large invasive cribriform carcinoma also occurred more often in Gleason score 4+4 than in 3+5 (61% *versus* 8%, P<0.001) or 5+3 (61% *versus* 22%, P<0.001) tumours, while its appearance in Gleason score 3+5 and 5+3 was not significantly different (P=0.08) in this cohort.

Gleason score 8 prostate cancer was stratified based on presence of invasive and/or intraductal cribriform carcinoma (Table 6.2). Non-organ confined disease (63% *versus* 34%, ≥pT3a, P=0.003) and positive pelvic lymph nodes (19% *versus* 3%, P=0.05) were more common in patients with cribriform architecture. Age, PSA levels and surgical margin status were not significantly different between Gleason score 8 patients with or without cribriform architecture. Patients with cribriform architecture had significantly shorter biochemical recurrence-free (log-rank P=0.001), metastasis-free (log-rank P<0.001) and disease specific (log-rank P=0.01) survival than those without (Figure 6.3).

Histomorphology of Gleason pattern 5

Gleason pattern 5 was observed in 110 (79%) Gleason score 8 tumours. In addition to men with Gleason score 3+5 and 5+3, 16 (35%) men with Gleason score 4+4 had tertiary Gleason pattern 5. Single cells and/or cords were present in 99/110 (90%) and solid fields in 32/110 (29%) tumours. All Gleason 5 patterns were simultaneously present in 26 (24%) cases. Invasive and/or intraductal cribriform carcinoma was present in 23/32 (72%) cases with solid pattern 5 and in 36/78 (46%) cases with non-solid pattern 5 (P=0.02). Of interest, the nine solid field cases without associated cribriform architecture all were of the small nested type (Figure 6.1, D).

Table 6.2. Gleason score 8 patients stratified for presence of cribriform architecture.

	Cribriform negative n=53	*Cribriform positive n=87*	*P-value*
Age (years)	63.7 (64.1; 59.7-67.4)	65.4 (66.1; 62.7-69.2)	0.09
PSA (ng/ml)	12.5 (10.0; 7.0-14.0)	13.2 (10.0; 7.5-16.0)	0.33
pT-stage			
T2	35 (66%)	32 (37%)	0.003
T3a	10 (19%)	34 (39%)	
T3b/T4	8 (15%)	21 (24%)	
Gleason pattern 4	45 (85%)	86 (99%)	<0.001
Small cribriform	0	83 (95%)	<0.001
Large cribriform	0	38 (44%)	<0.001
Intraductal carcinoma	0	48 (55%)	<0.001
Gleason pattern 5	50 (94%)	60 (69%)	<0.001
Single cells and/or cords	48 (91%)	51 (59%)	<0.001
Small solid nests	10 (19%)	13 (15%)	0.54
Medium to large solid fields	0	15 (17%)	<0.001
Comedonecrosis	0	9 (10%)	0.02
Positive surgical margins	25 (47%)	43 (49%)	0.80
Pelvic lymph node dissection	32 (60%)	59 (68%)	0.37
Lymph node metastasis	1 (3%)	11 (19%)	0.05
Biochemical recurrence	16 (30%)	52 (60%)	0.001
Metastasis	4 (8%)	32 (37%)	<0.001
Disease-specific death	0	12 (14%)	0.004

Values denote either mean (median; IQR) or n (%). PSA = Prostate Specific Antigen.

Comedonecrosis was present in 9 cases, 7 (78%) of which were present in Gleason score 4+4 tumours. Comedonecrosis was accompanied by cribriform architecture in all 9/9 (100%) cases and by solid fields in 5/9 (56%) cases. Moreover, comedonecrosis was observed more often in patients with large cribriform fields (7/20, 35%) than in those without (2/90, 2%, P<0.001).

Multivariable analysis of clinical outcome in Gleason score 8 patients

In univariate Cox regression analysis, pT3a (Hazard Ratio (HR) 2.1, 95% Confidence Interval (CI) 1.1-3.8, P=0.02), pT3b/4 (HR 4.6, 95% CI 2.5-8.5, P<0.001), Gleason score 4+4 (HR 1.9, 95% CI 1.1-3.1, P=0.02), positive lymph nodes at time of operation (HR 11.8, 95% CI 5.6-25.2, P<0.001) and overall presence of invasive and/or intraductal cribriform carcinoma (HR 2.4, 95% CI 1.4-4.1, P=0.003) were significantly associated with shorter biochemical recurrence-free survival, while age (P=0.18), PSA level (P=0.43), Gleason score 5+3 (P=0.78) and surgical margin status (P=0.21) were not (Table 6.3). In multivariable analysis, pT3b/4-stage (HR 4.4, 95% CI 2.1-9.3, P<0.001), positive lymph nodes (HR 9.9, 95% CI 4.2-23.5, P<0.001) and overall cribriform architecture (HR 2.0, 95% CI 1.0-3.7, P=0.04) had independent predictive value for biochemical recurrence-free survival, while Gleason score 4+4 (HR 1.7, 95% CI 1.0-2.9, P=0.07) did not meet conventional measures of significance in this cohort. In case individual cribriform growth patterns were included in multivariable analysis instead of overall cribriform architecture, large invasive cribriform carcinoma (HR 2.0, 95% CI 1.0-4.1, P=0.05) had independent predictive value for biochemical recurrence-free survival, whereas intraductal cribriform carcinoma (HR 1.3, 95% CI 0.8-3.5, P=0.4) and small invasive cribriform carcinoma (HR1.6, 95% CI 0.8-3.5, P=0.2) did not (*data not shown)*.

Similar trends were observed for metastasis as pT3a (HR 2.7, 95% CI 1.2-6.2, P=0.02), Gleason score 4+4 (HR 3.8, 95% CI 1.8-8.1, P=0.001), positive lymph nodes (HR 11.5, 95% CI 5.2-25.9, P<0.001) and overall cribriform architecture (HR 6.7, 95% CI 2.0-21.9, P=0.002) were significantly associated with shorter metastasis-free survival, whereas age (P=0.80), PSA level (P=0.96), pT3b/4 (P=0.06), Gleason score 5+3 (P=0.85) and positive surgical margins (P=0.95) were not (Table 6.4). In multivariable analysis, Gleason score 4+4 (HR 2.4, 95% CI 1.0-5.9, P=0.05), positive lymph nodes (HR 15.0, 95% CI 5.6-40.0, P<0.001) and overall cribriform architecture (HR 3.5, 95% CI 1.0-12.3, P=0.05) had independent predictive value for metastasis-free survival. Due to the low number of events and risk of model overfitting we were not able to include individual cribriform or Gleason 5 growth patterns in multivariable analysis.

Table 6.3. Cox regression analysis for biochemical recurrence-free survival in Gleason score 8 patients.

	Univariate			Multivariable		
	HR*	95% CI	P-value	HR	95% CI	P-value
Age (years)	1.0	0.9-1.0	0.18	1.0	0.9-1.0	0.05
PSA (ng/ml)	1.0	1.0-1.0	0.43	1.0	1.0-1.0	0.23
pT-stage						
T2	*ref*			*ref*		
T3a	2.1	1.1-3.8	0.02	1.8	1.0-3.4	0.06
T3b/T4	4.6	2.5-8.5	<0.001	4.4	2.1-9.3	<0.001
Gleason score						
3 + 5	*ref*			*ref*		
4 + 4	1.9	1.1-3.1	0.02	1.7	1.0-2.9	0.07
5 + 3	1.1	0.5-2.4	0.78	1.5	0.6-3.6	0.37
Positive surgical margins	1.4	0.8-2.2	0.21	0.8	0.5-1.5	0.55
Pelvic lymph node metastasis	11.8	5.6-25.2	<0.001	9.9	4.2-23.5	<0.001
Cribriform architecture	2.4	1.4-4.1	0.003	2.0	1.0-3.7	0.04

*HR = hazard ratio, CI = confidence interval.

Table 6.4. Cox regression analysis for metastasis-free survival in Gleason score 8 patients.

	Univariate			Multivariable		
	HR*	95% CI	P-value	HR	95% CI	P-value
Age (years)	1.0	0.9-1.1	0.80	1.0	0.9-1.1	0.52
PSA (ng/ml)	1.0	1.0-1.0	0.96	1.0	0.9-1.0	0.50
pT-stage						
T2	*ref*			*ref*		
T3a	2.7	1.2-6.2	0.02	2.5	1.0-6.7	0.06
T3b/T4	2.4	1.0-6.0	0.06	1.3	0.4-3.8	0.67
Gleason score						
3 + 5	*ref*			*ref*		
4 + 4	3.8	1.8-8.1	0.001	2.4	1.0-5.9	0.05
5 + 3	1.1	0.32-4.0	0.85	0.7	0.2-3.0	0.65
Positive surgical margins	1.0	0.5-2.0	0.95	1.1	0.5-2.4	0.84
Pelvic lymph node metastasis	11.5	5.2-25.9	<0.001	15.0	5.6-40.0	<0.001
Cribriform architecture	6.7	2.0-21.9	0.002	3.5	1.0-12.3	0.05

*HR = hazard ratio, CI = confidence interval.

Discussion

Our study demonstrates that among Gleason score 8 prostate cancer patients on radical prostatectomy, biochemical recurrence and metastases occur more often in Gleason score 4+4 than in Gleason score 3+5 or 5+3 tumours. Invasive and/or intraductal cribriform carcinoma was observed in 62% of tumours and was associated with adverse pathological features and clinical outcome. Cribriform architecture occurred more frequently in men with Gleason score 4+4 (93%) than in those with Gleason score 3+5 (47%) and 5+3 (44%). In multivariable analysis, cribriform architecture was an independent parameter for biochemical recurrence- and metastasis-free survival, while Gleason score was not. Therefore, cribriform architecture also has important value for risk stratification among Gleason score 8 prostate cancer patients.

Since the introduction of Grade Groups several reports have analysed the clinical outcome of Gleason score 3+5, 4+4 and 5+3 prostate cancer.[98] Some of these studies found that men with Gleason score 3+5 at radical prostatectomy had reduced risk of biochemical recurrence among Gleason score 8 patients. [104, 105] Others did not find a difference among Gleason score 8 subgroups or concluded that men with primary Gleason pattern 5 had worse outcome.[100, 106-109] This variability of results might be explained by the use of different specimen types and clinical outcome measures.[110] Furthermore, Gleason score 8 is relatively uncommon, hampering statistical analysis on large numbers of patients or resulting in clustering of Gleason score 5+3 and 5+3 tumours.[109, 111] Finally, from a morphologically point of view, Gleason score 8 prostate cancer is a very heterogeneous disease including highly variable quantities of Gleason 3, 4 and 5 growth patterns. This heterogeneity might lead to significant interobserver variability in tumour grading. For instance, Shah et al. only found fair interobserver reproducibility for Gleason pattern 5 assignment among 16 international expert genitourinary pathologists.[112] Upon re-review of 40 archival cases with Gleason score 5+3 prostate cancer, Kryvenko et al. assigned the same score in only 4 (10%) specimens, but upgraded 57.5% and downgraded 17.5% of cases.[113]

Many studies demonstrated worse clinical outcome for patients with cribriform architecture.[13, 15, 16, 28, 34] Most of these studies investigated cribriform architecture in intermediate grade prostate cancer, while the impact of cribriform architecture in Gleason score ≥4+3 is less well established. In Gleason score 7-10 prostate cancer biopsy patients, presence of cribriform architecture has been associated with advanced pathological stage and worse disease-specific survival compared to those with cribriform-negative biopsies.[39, 59] Harding-Jackson et al. found cribriform architecture, but not Gleason score, to have independent predictive value for cancer-specific survival in Gleason score 8 patients.[100] In the current study, we confirmed that cribriform architecture had strong discriminative value, even in aggressive Gleason score 8 prostate cancer.

Both overall invasive cribriform and/or intraductal carcinoma as well as its more aggressive large cribriform variant were significantly more often observed in Gleason score 4+4, than 3+5 and 5+3 disease. Since its association with adverse outcome, the high frequency of cribriform architecture might well explain the worse outcome of Gleason score 4+4 prostate cancer compared to those with 3+5 in our study, while the low number of 5+3 patients hampered powerful statistical analysis. Of interest, however, is that in multivariable analysis not only cribriform architecture but also Gleason score 4+4 had independent prognostic value for metastasis-free survival. A similar trend was observed for biochemical recurrence-free survival although the predictive value of Gleason score 4+4 did not reach conventional measures of significance (P=0.07). This implicates that other grading factors apart from cribriform architecture contribute to the worse outcome in Gleason score 4+4 patients. A possible explanation could be that Gleason score 4+4 disease has the lowest percent of Gleason 3 growth pattern, which is by definition present in less than 5% of the tumour volume. In 3+5 disease, percent Gleason pattern 3 theoretically varies from 50% to 95%, while it occupies 5% to 50% in Gleason score 5+3 tumours. Some groups have shown independent prognostic value for percent Gleason pattern 4 and 5, which outperformed Gleason score.[114] The inverse could well be true for percent Gleason pattern 3; if a tumour still has the biological capacity to mature into well-delineated glandular structures it is associated with better outcome.

Little is known about the predictive value of individual Gleason grade 5 growth patterns, which have been reported as either single cells, cords, small solid cylinders, solid fields, and presence of comedonecrosis.[12] Single cells and/or cords are the most common Gleason pattern 5.[115, 116] Flood et al. found that presence of solid fields and number of different Gleason 5 growth patterns were associated with shorter biochemical recurrence-free survival in Gleason score 9-10 prostatectomies.[115] Compared to other Gleason 5 patterns, comedonecrosis was associated with non-organ confined disease and biochemical recurrence.[103, 117] While individual Gleason 4 growth patterns have increasingly been subject to clinicopathological analysis, information on the clinical relevance of Gleason 5 patterns is still scarce. Our group recently performed in-depth three-dimensional visualization of prostate adenocarcinoma architectural growth patterns and revealed two separate morphological groups.[96] The first group consists of a tubular network in which the vast majority if not all tumour cells are in direct contact with surrounding stroma. This group encompasses the morphological continuum of Gleason pattern 3, poorly formed and fused pattern 4, and single cells and cords pattern 5. The second group has contiguous epithelial proliferations in which the majority of tumour cells are not in contact with surrounding stroma and consists of cribriform pattern 4 and solid pattern 5 with or without comedonecrosis. Our current finding that solid fields mostly coexisted with cribriform structures is reflective of this continuum. In the

current study, we distinguished between small nested cylinders consisting up to 30 tumour cells and larger solid fields. While the latter was continuous with cribriform growth, small nested cylinders were not. This suggests that both have different biological and possibly clinical relevance. However, larger studies are required to perform statistical analysis on the clinical relevance of individual Gleason 5 growth patterns.

Strong points of this study are the detailed histological review of radical prostatectomy specimens and the classification of cribriform architecture with the use of strict morphological criteria and additional immunohistochemistry. The study is limited by the retrospective study design. The inclusion of high-grade samples from two participating centers could have resulted in a selection bias. Furthermore, the relatively short follow-up of 59 months and limited number of patients restricted robust statistical analysis.

In conclusion, Gleason score 8 is a heterogeneous group of prostate cancers. Although clinicopathological characteristics of Gleason score 3+5, 4+4 and 5+3 are mostly similar, Gleason score 4+4 patients have a higher risk of adverse events. Cribriform architecture is an independent predictor for metastasis-free survival and has better discriminative value for clinicopathological outcome than Gleason score. Therefore, reporting cribriform architecture might add value in risk stratification of Gleason score 8 prostate cancer patients.

Chapter VII

Discussion.

Adapted from 'Prostate cancer growth patterns beyond Gleason score: entering a new era of comprehensive tumour grading'

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Histopathology. 2020 Dec; 77: 850-861.

Introduction

Prostate cancer management depends on a multitude of parameters for optimal risk stratification. Prostate-Specific Antigen (PSA) level, clinical stage and histology of prostate biopsies are key components in predictive risk models and subsequent clinical decision-making. The Gleason grading system has important value for determining prostate cancer prognosis and clinical decision-making. The Gleason score is entirely based on classification of architectural growth patterns. These basic patterns are assigned a Gleason grade from 1 to 5. Since prostate cancer is such a heterogeneous disease, the Gleason score is determined by adding to the most common and highest grade in biopsies, or two most predominant grades in radical prostatectomy (RP) specimens.[11, 118] Gleason pattern 1 to 3 encompass well-delineated glandular structures with variable inter-glandular distance and nodular circumscription. As no practical nor prognostic differences exist between these three Gleason grades, the International Society of Urological Pathology (ISUP) recommended that Gleason scores 2-4 should rarely, if ever, be used on biopsy specimens.[11] Gleason pattern 4 includes poorly formed, fused, glomeruloid and cribriform glandular structures, whereas growth patterns with essentially no glandular differentiation such as single cells, cords, solid fields and presence of comedonecrosis classify as Gleason pattern 5 (Figure 1).[11, 12] While individual growth patterns have not been specified in pathology reporting, clinical decision-making or molecular-biological investigations, recent studies indicate that individual growth patterns have independent predictive value for clinical outcome and facilitate more comprehensive interpretation of molecular-biological findings. The aim of this Discussion is to summarize the clinicopathological impact of individual prostate cancer growth patterns beyond Gleason score and to investigate their molecular-biological background. Moreover, we aim to give recommendations on the incorporation of architectural growth patterns in order to optimize decision-making in clinical practice.

Clinicopathological impact of cribriform growth patterns and their variants

Individual tumour growth patterns have mainly been analysed in Gleason score 3+4=7 (Grade Group 2) prostate cancer, which is composed of variable quantities of well-delineated Gleason pattern 3 glands and Gleason pattern 4 structures. In 2011, Iczkowski et al. were the first to report that cribriform growth pattern had independent prognostic value for post-operative PSA failure.[13] Many groups have thereafter confirmed the independent predictive value of cribriform pattern for adverse pathological features, biochemical recurrence- and disease specific-free survival in biopsy and RP specimens.[14, 15, 20, 21, 34, 39, 52, 67, 71, 119, 120] However, a caveat of many studies on cribriform growth pattern is that it is not entirely clear whether, and if so how, invasive Gleason pattern 4 pattern was distinguished from intraductal carcinoma (IDC) of the prostate. IDC is characterized

by a cribriform or solid proliferation of atypical epithelial cells within distended pre-existent prostate acini either with or without comedonecrosis, and has also been associated with adverse clinical outcome.[28, 36, 39, 52, 121-123] The aetiology of IDC is not yet elucidated. While IDC is thought to represent cancerisation of pre-existent glands by an invasive carcinoma, some data suggests that IDC arises as a precursor lesion that has progressed beyond high grade prostatic intraepithelial neoplasia (hgPIN).[25] Invasive cribriform carcinoma and IDC are often difficult, if not impossible, to distinguish without the application of basal cell immunohistochemistry. If no basal cells are present, a cribriform lesion is generally considered as invasive Gleason pattern 4; if continuous, scattered or sporadic basal cells are observed, cribriform architecture is mostly regarded as IDC. Only few studies have attempted to investigate invasive cribriform and intraductal carcinoma separately by using extensive immunohistochemistry.[34, 39, 51] In a prostate biopsy screening cohort of 1,031 men, Kweldam et al. found that presence of invasive cribriform carcinoma and IDC were both associated with worse disease-specific survival in univariate analyses. The combination of either invasive cribriform or intraductal carcinoma showed the strongest association with outcome in this study. In the vast majority of patients, IDC occurs intermixed with invasive carcinoma, but rare cases of isolated IDC without invasive disease have been described.[24]

Grading of IDC intermixed with invasive carcinoma has been controversial. While the 2014 ISUP meeting did not make a recommendation on this issue, the 2016 WHO states it should not be factored into grading.[12, 118] A consequence of the WHO recommendation is that basal cell immunohistochemistry should be performed in every case where IDC cannot be distinguished from invasive disease and classification of atypical lesion as either IDC or invasive carcinoma would alter the final Gleason score. Apart from additional run-around time and costs, basal cell immunohistochemistry does not distinguish between invasive cribriform or solid carcinoma, and IDC in every case. It is well-known that foci of hgPIN can lack basal cells probably due to sampling artifact. As IDC glands are by definition distended, the chance of false-negative basal cell immunohistochemistry seems even larger, resulting in erroneous classification as invasive cribriform pattern. On the other hand, large irregular cribriform tumour fields well exceeding pre-existent gland architecture might have rare basal cells, as has also rarely been reported for low-grade invasive adenocarcinoma.[124] Since IDC is an adverse pathological parameter and difficult or even impossible to distinguish from invasive carcinoma, even with the use of basal cell immunohistochemistry, it is recommended by the latest 2019 ISUP consensus meeting that IDC intermixed with invasive carcinoma should be assigned a Gleason grade based on its underlying growth pattern, as if it were invasive carcinoma.[125] This incorporation of intraductal carcinoma into the tumour grade results in a Grade Group change in less than 2% of prostate cancer

biopsies.[126] Although the modification might affect decision-making in individual patients, it has minimal impact on overall prostate cancer management.

With increasing awareness of the their clinical impact, proposals for subclassification of cribriform growth patterns have been made. More detailed analysis of cribriform pattern has shown that the number of cribriform fields does not seem to affect clinical outcome in a negative way, while their maximal individual size does.[34, 39, 51] In the current thesis, , we found in 420 Grade Group 2 RPs that large expansile cribriform fields, arbitrarily defined as exceeding at least two times the size of adjacent benign glandular structures, had seminal vesicle invasion in 32% and pelvic node metastasis in 23% of cases, which was significantly higher than 9% and 5% found, respectively, for small invasive cribriform carcinoma.[51] Other groups have also separated both cribriform patterns, but are difficult to compare as they applied other size criteria such as presence of at least 12 intercellular lumens or exceeding average benign gland diameter.[13, 34, 127] Therefore, we concluded that large cribriform carcinoma was an even more aggressive subtype.

Clinical implications of incorporating cribriform growth patterns

Since they both have independent predictive value, the presence of either invasive cribriform or intraductal cribriform carcinoma should routinely be reported as "cribriform carcinoma".[125] The question arises as to what extent incorporation of cribriform carcinoma can lead to optimization of therapeutic decision-making in individual prostate cancer patients. Patients with biopsy Gleason score 3+4=7 (Grade Group 2) prostate cancer will generally be offered definitive treatment, while those with Gleason score 3+3=6 (Grade Group 1) are often eligible for active surveillance. Recent identification of additional prognostic pathological parameters such as presence of invasive and/or intraductal cribriform carcinoma and Gleason pattern 4 quantity, allows for more detailed risk stratification of men with Grade Group 2 disease. Men with low biopsy Gleason pattern 4 quantity might be eligible for active surveillance, firstly because their outcome is comparable to those with Grade group 1 tumours, secondly due to the substantial inter-observer variability for grade assignment to small foci of poorly formed and fused glands.[43, 44, 128-131] Since disease-specific and biochemical recurrence-free survival are not statistically significantly different among men with biopsy Grade Group 2 without cribriform carcinoma and those with Grade Group 1, absence of both invasive cribriform and intraductal carcinoma has also been proposed as an eligible criterion for active surveillance for men with Grade Group 2 biopsies.[39, 48, 52, 132] If the safety of this eligibility approach is shown in prospective studies, this will have major impact on individual Grade Group 2 patient management. Presence of invasive carcinoma might also affect other aspects of clinical decision-making. Absence of cribriform architecture has been associated with a low-risk of pelvic lymph node metastasis.[33, 120, 133] In our series of 627 RP's,

22/228 (10%) Grade Group 2 men with cribriform carcinoma developed metastases, while no metastases were observed among 192 cribriform-negative Grade Group 2 and 207 Grade Group 1 patients (this thesis).[133] Although higher biochemical recurrence rates were seen in men with Grade Group 2 prostate cancer, this did not affect long term outcome in our cohort. This indicates that invasive cribriform and intraductal carcinoma, in particular, might have an impact on the biological potential for metastatic disease to develop. In contrast, postoperative biochemical recurrence-free survival is also related to tumour volume parameters and surgical technique, which do not necessarily reflect biological derangement caused by the disease.

Current guidelines on performing of pelvic lymph node dissections (PLND) are based on clinicopathological nomograms which do not consider cribriform architecture, but future inclusion of invasive cribriform and intraductal carcinoma might result in optimization of these nomograms. Few studies also have found independent value of cribriform carcinoma for response to radiation therapy and docetaxel, but the definitive clinical impact on these treatment modalities remains to be determined.[121, 134, 135]

Detection of cribriform growth patterns with MRI and prostate biopsies

Since invasive cribriform and intraductal carcinoma might increasingly affect clinical decision-making, the sensitivity for detection of these adverse features on biopsy should ideally be high. Concordance between Grade Group at RP specimens and matched biopsies is relatively low, with tumour up-grading occurring in up to 40% of cases.[94, 136] The sensitivity and specificity for identification of invasive and/or intraductal cribriform carcinoma at biopsy specimens varies from 43-56% and 87-95%, respectively.[59, 137, 138] This indicates that about half of cribriform carcinoma lesions are missed at diagnostic biopsies. Detailed analysis of features potentially associated with cribriform false-negative biopsies, did not reveal predictive value for number of positive biopsies, percent Gleason pattern 4, or presence of glomeruloid Gleason pattern 4 architecture in a relatively small series performed in this thesis.[138] On the other hand, multiparametric magnetic resonance imaging (MRI) might have added value in identification of men with prostatic invasive cribriform and intraductal carcinoma with false-negative biopsies, since these more frequently present with PI-RADS score 5 lesions.[49, 138, 139] Finally, commercially available molecular tests might also play a role in identification of men with these adverse features as discussed in more detail later.[140-142]

Invasive cribriform growth pattern delineation

As invasive cribriform carcinoma should be separately commented on in pathology reports and might increasingly affect individual therapeutic decision-making, it is important to gain broad consensus on the definition of cribriform morphology and to delineate this growth pattern from its microscopic mimickers.[65] Recognition and Gleason pattern 4 assignment is better for cribriform and glomeruloid growth pattern than for poorly-formed and fused glands.[94, 95, 131] Nevertheless, tangentially sectioned tumour glands, complex fused glands, large glomeruloid structures and solid Gleason pattern 5 might all be confused with invasive cribriform pattern.[95] Our group has defined cribriform architecture as an epithelial sheet (a) where the majority of tumour cells do not contact surrounding stroma, (b) with a polarized gland-like structure being present at less than half of the sheet circumference and (c) with regular intercellular lumens clearly visible on HE section (Figure 2).[65, 143] The first criterion (a) distinguishes cribriform from complex fused glands where most if not all tumour cells are still in direct contact with subtle connective tissue cores being present within the lesion. The second criterion (b) arbitrarily distinguishes cribriform from large glomeruloid pattern where polarized gland-like spaces are circumventing more than half of the central protrusion. The validity of this criterion is supported by the fact that clinicopathological features and biochemical recurrence-free survival of large glomeruloid structures at RP were more comparable to those of small glomeruloid than cribriform Gleason pattern 4 in our study (this thesis).[144] The third criterion (c) distinguishes cribriform from solid Gleason pattern 5 where essentially no glandular differentiation is visible on HE sections.

Clinicopathological relevance of other growth patterns

Whereas invasive cribriform and intraductal carcinoma have independent value for clinical outcome in men with Grade Group 2 prostate cancer, it is not entirely clear yet to what extent outcome of Grade Group 2 men without cribriform architecture differs from those with Grade group 1 tumours. With a median follow-up of 13 years, Kweldam et al. did not find statistically different disease-specific survival for 256 patients with biopsy cribriform-negative Grade group 2 and 486 Grade group 1 prostate cancer.[52] In our RP cohort, we did not find any metastasis at PLND or during follow-up in207 men with Grade Group 1 and 197 Grade Group 2 cancer without invasive cribriform or intraductal carcinoma.[133] The latter group, however, had significantly shorter biochemical recurrence-free survival than men with Grade Group 1. These data suggest that cribriform architecture is reflective of intrinsic capacity for development of metastasis, while the risk of biochemical recurrence also more depends on other factors such as tumour volume, positive surgical margins on non-cribriform Gleason patterns. In a detailed study of 1275 RPs, McKenney et al. distinguished 20 different growth pattern features.[21] Apart from the adverse

outcome of cribriform architecture compared to poorly-formed glands, they revealed reactive stroma response to be associated with worse and mucin extravasation with better prognosis.

Hitherto, the glomeruloid growth pattern has not yet been adequately addressed in literature. In 1995, glomerulations were described for the first time in prostate needle biopsies.[145] Glomeruloid growth consists of carcinoma glands with intraluminal cell clusters or cribriform structures, attached to one side of the gland wall, resembling a glomerulus.[12] Pacelli et al. were the first to describe the glomeruloid growth pattern in relation to higher tumour grade and pathological stage.[92] Lotan et al. observed the glomeruloid growth pattern often simultaneously with high tumour grade and especially cribriform growth.[19] Because of its morphological reminiscence and frequent co-occurrence with cribriform pattern, glomeruloid architecture has been classified as Gleason pattern 4 since the 2014 ISUP consensus meeting, and some authors have postulated it to represent a precursor of cribriform morphology.[12, 19, 20] In 350 RP specimens with Gleason score 7, Choy et al. however found that cribriform morphology independently increased the risk of biochemical recurrence, while glomeruloid architecture was significantly associated with a reduced risk.[20] The previously mentioned study by McKenney et al. could not associate glomerulations with adverse recurrence-free survival.[21] In our study among 472 Grade group 2 RPs, we distinguished small simple glomerulations and complex glomerulations with cribriform protrusions (this thesis).[144] Complex glomerulations coincided with cribriform architecture more often than simple glomerulations. Men with cribriform morphology had significantly worse clinicopathological features and biochemical recurrence-free survival than those with glomeruloid pattern, irrespective of the size of the glomerulations. These findings seem counterintuitive with the hypothesis that glomeruloid glands are precursors of cribriform architecture.

Still little is known on the clinical relevance of Gleason 5 growth patterns, which have been classified as single cells, cords, solid fields, or presence of comedonecrosis.[118] This is mostly due to the fact that tumours with primary, secondary or tertiary Gleason pattern 5 are very heterogeneous with variable quantities of Gleason pattern 3 , 4 and 5, several different growth patterns and occurrence of IDC. Meaningful statistical analysis including all relevant covariates requires inclusion of a large number of these patients. In that sense, it is of interest that even in Grade Group 4 patients, presence of cribriform architecture has independent predictive value for biochemical recurrence-free and disease-specific survival.[39, 100, 146] In our cohort, presence of cribriform architecture outperformed Gleason score in predicting outcome regarding biochemical recurrence- and metastasis-free survival (this thesis).[146] Nevertheless, presence of comedonecrosis and solid sheets were found to be adverse parameters amongst Gleason 5 patterns in two relatively small series.[115, 117]

Molecular aberrations in cribriform architecture

Genomic features of prostate cancer differ from other cancers, firstly due to the relatively low mutation rate in prostate cancer. It features copy number variations involving prostate oncogenes and tumour suppressor genes, and continuous, accumulating genomic and epigenetic alterations.[147, 148] The *TMPRSS2-ERG* fusion is one of the most common alterations, seen in 50% of prostate cancers, and is associated with low-grade tumours.[149] The androgen receptor pathway is a key element in prostate cancer progression and harbours alterations in most castration-resistant prostate cancers.[150] In addition, gains of oncogene *c-MYC*, loss of tumour suppressor gene *PTEN* and mutations in the *SPOP* gene have been associated with aggressive disease.[151-155]

Molecular alterations in prostate cancer have mostly been examined by Gleason score without taking underlying growth patterns into account. Recently, few groups have aimed to identify molecular characteristics of invasive cribriform and intraductal carcinoma.[18, 78, 79] Since these bioinformatic analysis were performed retrospectively on publically available databases with digitally scanned HE reference slides, no reliable distinction between invasive and intraductal cribriform carcinoma could be made. Cribriform carcinoma is characterised by genomic instability and has been posited to be the result of an accumulation of aggressive features above early phase tumour alterations within the prostate epithelium.[18, 78] Amongst others, deletions of 8p, 10q and amplification of 8q24 corresponding to *PTEN* loss and *c-MYC* gain were significantly enriched in cribriform carcinoma, together with *SPOP* point-mutations.[18, 78, 79, 156, 157] Molecular profiling and RNA in situ hybridization revealed that the long noncoding RNA SChLAP1 had more than 3-fold higher levels in cribriform architecture, and could serve as a potential marker for its detection in clinical practice.[78, 158] Epigenetic alternations have been detected in cribriform prostate cancer as well. The hypermethylated clusters found in prostate cancer are enriched by cribriform patterns, while non-cribriform patterns dominate the hypomethylated cluster.[79, 159, 160] Interestingly, various of the molecular aberrations associated with cribriform carcinoma have been linked to aggressive clinical behaviour of prostate cancer before.[161-166] Moreover, distant metastasis were found to be partially comparable to intraductal cribriform carcinoma.[80] Together these data indicate that cribriform carcinoma is a morphological substrate of increased genomic instability, and brings histopathology, molecular aberrations and adverse clinical outcome comprehensively together.

Molecular identification of a "cribriform signature" is of importance, especially since the high rate of false cribriform-negative biopsies is a limitation for developing clinical decision models incorporating cribriform carcinoma. A clinically applicable molecular urine, serum or tissue test might identify men at risk for unsampled cribriform carcinoma. Recently, studies have shown that higher risk scores using RNA expression-based tissue arrays (Decipher, Oncotype Dx and Prolaris) were significantly associated with presence of cribriform carcinoma in the tissue sample

analysed.[140, 141, 167] It remains to be determined whether these molecular assays still have added clinical value if cribriform carcinoma and percent Gleason pattern 4 are accounted for, and if they can support identification of men with false cribriform-negative prostate cancer biopsies.

Three-dimensional architecture of prostate cancer growth patterns

Microscopic diagnostic pathology in everyday practice is performed using thin tissue slides representing two-dimensional cross-sections of a three-dimensional (3D) structure. Little is known on the actual 3D architecture of prostate carcinoma growth patterns. Registration of hundreds of consecutive slides have shown that poorly-formed Gleason pattern 4 is continuous with Gleason pattern 3.[168, 169] Recent improvements in tissue clearing techniques, long-distance confocal laser scanning and light-sheet microscopy have enabled imaging of intact 1 mm thick prostate tissues.[170-172] By detailed 3D analysis of formalin-fixed paraffin-embedded RP specimens, we were able to get comprehensive insight of the 3D architecture of prostate adenocarcinoma growth patterns.[143] This revealed that Gleason pattern 3 three-dimensionally represented tubules with local interconnections. This pattern was continuous with both poorly formed Gleason pattern 4 where tubular size and lumen diameter was smaller and tubular interconnections occurred more frequently, and fused pattern 4 where interconnections often occurred at distances smaller than the tubular diameter. In fact, single cells and cords Gleason pattern 5 formed a continuum with poorly formed glands, in which lumen size further decreased until its disappearance. On the other hand cribriform Gleason pattern 4 and solid pattern 5 either without or with comedonecrosis consisted of serpentine fields of epithelial cells with the majority of tumour cells not being in direct contact with surrounding stroma. Both patterns formed a continuum with or without the presence of recognizable intercellular lumens. Based on these three-dimensional features, we classified the growth patterns in two distinct subgroups which both formed continua. The first group consisted of Gleason pattern 3 tubules, Gleason pattern 4 poorly formed and fused glands, and Gleason pattern 5 single cells and cords, which all consisted of cells directly contacting surrounding stroma, but with variable gland size, lumen size and number of interconnections. The second group encompassed cribriform Gleason pattern 4 and solid pattern 5 without or with comedonecrosis consisting of epithelial cells, where the majority of cells did not connect to adjacent stroma and with variable intercellular lumen frequency and size. Glomeruloid structures formed a 3D intermediate between both subgroups. They represented intraluminal protrusions of epithelial cells appearing within a background of Gleason pattern 3 tubules and were mostly present at the side of tubular interconnections. The 3D architectural continuity and transitions between growth patterns in both subgroups well explains Gleason grading inter-observer variability.[94, 136] Whereas growth patterns are ordinally classified in Gleason grades in clinical practice, they gradually

transition to each other without presence of clearly identifiable cut-offs. Furthermore, the 3D architectural relationships support the definition and delineating characteristics of cribriform growth pattern as mentioned previously. Future studies need to determine whether the 3D dichotomization is also reflected by clinical and molecular observations.

Digital image analysis for automated detection of cribriform growth pattern

Digitalization in the field of pathology offers advantages in cancer diagnosis with the use of deep learning algorithms.[173-175] In radiology, computer-aided detection of prostate cancer in multiparametric MRI images has similar performance scores as a radiologist, including to differentiate between low- and high-grade prostate cancer.[176, 177] For histological evaluation, deep learning systems can be designed to segment epithelial tissue and recognize malignant glands. In prostate needle biopsies, cancer likelihood maps have been computed with high sensitivity for carcinoma.[178] Hence, up to a third of slides not containing prostate cancer could be identified reliably and would not have to be evaluated by pathologists. Based on unforcedly generated features, deep learning systems are able to compute boundaries and cut-off points, thus enabling automated identification of histomorphological growth patterns.[178-180] As mentioned before, prostate cancer grading is prone to intra- and interobserver variability, while treatment options and outcome are highly dependent on assignment of patterns. By standardization, image analysis is able to reduce the variability and prioritize cases for the pathologist. In a collaborative study we focused on automated detection of cribriform growth in prostate cancer based on convolutional neural networks.[181] Original annotations performed by pathologists in prostate cancer biopsies showed that sensitive cribriform region detection can be reached, but at the expense of a high number of false positives.

Future perspectives

Last decade the growing interest in individual growth patterns in prostate cancer has resulted in identification of cribriform architecture as pathological factor with independent prognostic value.[65] However, the morphogenetic background of cribriform and other growth patterns remains to be elucidated. Furthermore, the relation of intraductal carcinoma and invasive cribriform carcinoma with show significant morphological overlap is yet unclear. To solve this issue, detailed molecular analyses of individually dissected growth patterns is mandatory. Furthermore cell culture and xenograft studies are subsequently important for investigating morphogenetic effects of specific molecular alterations.

In turn, molecular markers might contribute to detection and diagnosis of cribriform growth pattern in false negative biopsies. At this moment sensitivity of prostate cancer biopsies to

detect cribriform architecture is unsatisfactory, although the false negative rate is lower for the large cribriform subtype. In future studies it should be determined how multiparametric MRI and possibly commercially available molecular urine- or tissue-based tests could optimize the representability of prostate biopsies for the entire tumour.

Future multivariable analyses need to elucidate on the mutual interaction and independent value of the recently explored pathological parameters such as cribriform patterns and percentage Gleason pattern 4 and 5. After identification of the independent, most influential and reproducible factors, modification to the current Gleason grading/Grade group systems could even be made to increase the discriminative value of tumour grading.[53, 65] Furthermore, prospective studies including cribriform architecture in clinical decision-making for instance on eligibility for active surveillance, should indicate to what extent growth pattern specification can optimize patient management.

Conclusion

Presence of cribriform architecture is associated with dismal outcome with large cribriform architecture representing an even more aggressive subtype in prostate cancer. While glomerulations may harbour cribriform intraluminal protrusions, they had favourable outcome and should not be classified as a cribriform growth pattern variant. Treatment of patients with cribriform architecture is essential, but the sensitivity of cribriform growth pattern detection at biopsies should be improved. Incorporation of cribriform growth patterns beyond heterogeneous Gleason groups in pathology reporting and clinical decision-making will improve personalized treatment of prostate cancer patients.

Addendum

Summary

Prostate cancer is the most common cancer among men with great variability regarding outcome. Clinical decision-making depends on Prostate-Specific Antigen (PSA) levels, clinical tumour stage and Gleason score. The Gleason score is the most important parameter given by the clinical pathologist and is entirely based on tumour architectural growth patterns, which are classified into five different grades. The tumour is assigned a primary grade followed by a highest grade in needle biopsy or secondary grade in radical prostatectomy specimens. Men with Gleason score 3+3=6 (Grade Group 1) prostate cancer have excellent outcome and are frequently eligible for active surveillance. Men with Gleason score 3+4=7 (Grade Group 2) or higher generally receive radiation therapy or radical prostatectomy surgery. Despite the urge for active treatment, differences in clinical outcome are observed in intermediate to high risk prostate cancer patients. There is a need for additional parameters to aid risk stratification in these men. Incorporation of individual histopathological growth patterns might be able to support optimal decision-making in this large prostate cancer subpopulation.

Last decade the cribriform growth pattern has been recognized as independent prognostic pathological feature for prostate cancer outcome. Cribriform architecture however does not comprise one entity. **Chapter 2** elaborated on the prognostic impact of invasive and intraductal cribriform prostate cancer subtypes in Grade Group 2 radical prostatectomies. Cribriform architecture was associated with worse clinicopathological parameters such as tumour stage and lymph node metastasis. Of the 420 radical prostatectomies, 8% revealed invasive large cribriform architecture, based on a diameter of at least twice the size of adjacent pre-existent normal glands. Large cribriform architecture represented an aggressive subtype of invasive cribriform prostate cancer as it was an independent predictive factor for biochemical recurrence-free survival in patients with Grade Group 2 prostate cancer. Furthermore, metastases were more often seen in this cribriform subtype. If validated in biopsy cohorts, large cribriform pattern therefore could serve as a new predictive parameter for optimisation of clinical decision-making.

In order to incorporate cribriform architecture as predictive parameter, it is essential to detect it in diagnostic biopsies. In **Chapter 3**, we compared presence of cribriform architecture in biopsies and following radical prostatectomies. Patients had cribriform architecture on radical prostatectomy in 69% of cases, while on biopsies only 30% revealed this pattern. Of patients with Grade Group 2 prostate cancer, 40% had false negative biopsies for cribriform growth. These patients presented with higher PSA levels than men with true negative biopsies for cribriform growth and more often had radiological high grade lesions on magnetic resonance imaging. The false negative rate for large cribriform prostate cancer, the more aggressive cribriform subtype, was lower than for any

cribriform architecture. Also, biochemical recurrence-free was significantly worse for patients with true positive biopsies than false negative biopsies.

It has been well established that cribriform architecture is associated with dismal outcome, however absence of this pattern was less well studied. **Chapter 4** compared features and outcome of Grade Group 1 and Grade Group 2 prostate cancer without cribriform architecture. Although patients with cribriform-negative Grade Group 2 prostate cancer presented with higher PSA levels and higher frequency of extra-prostatic extension and positive surgical margins, this did not affect long-term outcome. Metastasis and disease-specific death occurred neither in patients with Grade Group 1 nor in cribriform-negative Grade Group 2. Biochemical recurrence-free survival was shortened in cribriform-negative Grade Group 2 patients, however this is also related to tumour volume parameters and surgical technique, which do not necessarily reflect biological derangement caused by the disease.

Glomeruloid growth is a hitherto poorly understood histomorphological pattern that bears similarities to cribriform architecture. Glomerulations with small intraluminal protrusions have been associated with favourable outcome. It is yet unclear what is the clinical behaviour of complex glomeruloid patterns, which resemble cribriform architecture. In **Chapter 5** we specifically aimed at characterization of glomeruloid architecture in radical prostatectomy specimens. Complex glomeruloid often coincided with other cribriform patterns. Although complex glomerulations were associated with slightly worse clinicopathological features, no independent prognostic value was seen for neither simple nor complex cribriform growth. Therefore, glomeruloid pattern and particularly complex glomerulations should not be classified as a cribriform growth pattern variant.

Chapter 6 focused on the effect of growth patterns in Grade Group 4 prostate cancer. Although the incorporation of growth patterns in prostate cancer diagnosis particularly affects clinical decision making in Grade Group 2 prostate cancer, it might also have predictive value in high-grade prostate cancer. We investigated presence of cribriform architecture in Grade Group 4 and found that it was present in 62% of cases. It was more frequently present in Gleason score 4 + 4 compared to 3 + 5 or 5 + 3 prostate cancer, and solid field pattern 5 often coexisted with cribriform architecture. Presence of cribriform architecture was an independent parameter for biochemical recurrence- and metastasis-free survival. Therefore, cribriform architecture also has added value in risk stratification of Gleason score 8 prostate cancer patients.

Finally, in **Chapter 7** the main findings of this thesis are summarized and reviewed together with current available knowledge on prostate cancer growth patterns. We also postulate how incorporation of cribriform architecture can optimize personalized clinical decision-making in prostate cancer patients.

Samenvatting

Prostaatkanker is de meest voorkomende kanker onder mannen, met grote variabiliteit in uitkomst. Klinische besluitvorming is afhankelijk van Prostaat-Specifieke Antigeen (PSA) levels, klinisch tumorstadium en Gleason score. De Gleason score is de belangrijkste door de klinisch patholoog afgegeven parameter en is gebaseerd op groeipatronen binnen de tumor, welke worden ingedeeld in vijf graden. Aan iedere tumor wordt een primaire graad toegekend, gevolgd door een hoogste graad in prostaatbiopten en secundaire graad in radicale prostatectomieën. Mannen met Gleason score 3+3 (Grade Group 1) prostaatkanker tonen excellente overleving en komen meestal in aanmerking voor actief vervolgen. Mannen met Gleason score 3+4 (Grade Group 2) of hoger ontvangen meestal behandeling in de vorm van radiotherapie of chirurgie. Ondanks de indicatie voor behandeling zijn er grote verschillen in klinische uitkomst tussen prostaatkanker patiënten uit deze intermediair tot hoog risico groep. Incorporatie van individuele histopathologische groeipatronen kan een additionele parameter in risicostratificatie zijn en optimale klinische besluitvorming faciliteren in deze grote prostaatkanker subpopulatie.

In het afgelopen decennium is cribriforme groeiwijze geïdentificeerd als onafhankelijke prognostische parameter bij prostaatkanker. Cribriforme architectuur omvat echter niet slechts één entiteit. **Hoofdstuk 2** onderzocht de prognostische waarde van subtypes van invasieve en intraductale cribriforme prostaatkanker in radicale prostatectomieën met Grade Group 2. Cribriforme architectuur was geassocieerd met ongunstige clinicopathologische parameters zoals hoog tumorstadium en lymfekliermetastasen. Van de 420 radicale prostatectomieën toonde 8% groot cribriforme groei, gebaseerd op een diameter van ten minste tweemaal die van een pre-existente buis. Groot cribriforme groei representeerde een agressief subtype cribriforme architectuur, daar het een onafhankelijke predictieve waarde had aangaande biochemisch recidief-vrije overleving in patiënten met Grade Group 2 prostaatkanker. Metastasen werden eveneens vaker gezien in dit cribriforme subtype. Wanneer deze parameter gevalideerd zou worden in een groot cohort, kan groot cribriforme groei een nieuwe predictieve parameter worden ter optimalisatie van klinische besluitvorming.

Om cribriforme architectuur en subtypes te incorporeren als predictieve marker, is het essentieel deze in diagnostische biopten op te sporen. In **hoofdstuk 3** vergeleken we de aanwezigheid van cribriforme architectuur op biopten en opvolgende radicale prostatectomieën. Patiënten toonden cribriforme architectuur in 69% van de radicale prostatectomieën, echter slechts 30% van de biopten bevatten dit patroon. Patiënten met Grade Group 2 prostaatkanker hadden fout-negatieve biopten voor cribriforme groei in 40% van de gevallen. Deze patiënten presenteerden zich met hoger PSA en hadden vaker een radiologisch hooggradige laesie op magnetic resonance imaging.

Het aantal fout-negatieve biopten lag echter lager voor patiënten met groot cribriforme groei, het agressieve subtype van cribriforme architectuur. Daarnaast was biochemisch recidief-vrije overleving significant slechter voor patiënten met waar-positieve dan fout-negatieve biopten.
Hoewel cribriforme architectuur geassocieerd is met een somberdere uitkomst, is de afwezigheid van dit patroon minder goed onderzocht. **Hoofdstuk 4** vergeleek de kenmerken en uitkomsten van Grade Group 1 en Grade Group 2 prostaatkanker zonder cribriforme architectuur. Ondanks het feit dat patiënten met cribriform-negatieve Grade Group 2 prostaatkanker hogere PSA levels en tumorstadium toonden, alsmede dat zij vaker positieve snijvlakken hadden, beïnvloedde dit niet de lange termijn uitkomsten. Metastasen en ziekte-specifiek overlijden kwamen niet voor in Grade Group 1 noch in cribriform-negatieve Grade Group 2 prostaatkanker. Biochemisch recidief-vrije overleving was korter in cribriform-negatieve Grade Group 2 patiënten, echter deze overleving is mede gerelateerd aan tumorvolume en chirurgische techniek, welke niet noodzakelijk samenhangen met biologisch gedrag van de tumor.
Glomeruloïde groei toont gelijkenissen met cribriforme architectuur en is tot dusverre weinig onderzocht. Glomerulaties met kleine intraluminale protusies zijn geassocieerd met betere prostaatkanker overleving. Het klinische gedrag van complexe glomeruloïde patronen, welke cribriform aspect kunnen tonen, is nog onduidelijk. **Hoofdstuk 5** richtte zich op karakterisatie van glomerulaties in radicale prostatectomieën. Complexe glomeruloïde groei werd vaak samen gezien met andere cribriforme patronen en was geassocieerd met enigszins minder gunstigere clinicopathologische kenmerken. Desalniettemin werd er geen onafhankelijke prognostische waarde gevonden voor simpele noch complexe glomeruloïde groei. Daarom dient glomeruloïde groei niet gekenmerkt te worden als subtype van cribriforme groei.
Hoofdstuk 6 onderzocht de groeipatronen in Grade Group 4 prostaatkanker. Het meewegen van groeipatronen in de prostaatkanker diagnose heeft misschien de meeste waarde voor klinische besluitvorming in Grade Group 2 patiënten, het kan eveneens aanvullende waarde tonen voor hooggradige prostaatkanker. Cribriforme architectuur werd onderzocht in Grade Group 4 prostaatkanker waar het in 62% van de gevallen voorkwam. Het werd vaker gezien in Gleason score 4+4 dan 3+5 of 5+3 prostaatkanker en vaak samen met solide groeipatronen. De aanwezigheid van cribriforme architectuur was een onafhankelijke parameter voor biochemisch recidief- en metastase-vrije overleving. Daarom heeft cribriforme architectuur toegevoegde waarde in de risicostratificatie van Gleason score 8 prostaatkanker patiënten.
Tenslotte worden in **hoofdstuk 7** de bevindingen van dit proefschrift uiteengezet en beoordeeld in het licht van huidige kennis over prostaatkanker groeipatronen. We betogen dat incorporatie van cribriforme architectuur de gepersonaliseerde klinische besluitvorming van prostaatkanker patiënten kan verbeteren.

List of Publications

Vos MC, Hollemans E, Ezendam N, Feijen H, Boll D, Pijlman B, van der Putten H, Klinkhamer P, van Kuppevelt TH, van der Wurff AA, Massuger LF. MMP-14 and CD44 in Epithelial-to-Mesenchymal Transition (EMT) in ovarian cancer. *J Ovarian Res.* 2016 Sep; 9: 53. doi: 10.1186/s13048-016-0262-7.

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Van Leenders GJLH, Kweldam CF, Hollemans E, Kümmerlin IP, Nieboer D, Verhoef EI, Remmers S, Incrocci L, Bangma CH, van der Kwast, TH, Roobol MJ. Improved prostate cancer biopsy grading by incorporation of invasive cribriform and intraductal carcinoma in the 2014 Grade Groups. *Eur Urol.* 2020 Feb; 77: 191-198 doi: 10.1016/j.eururo.2019.07.051.

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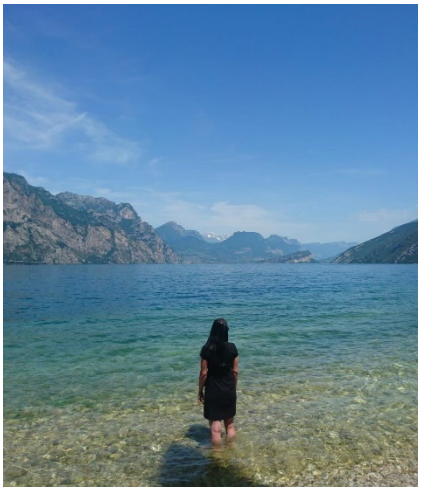
List of abbreviations

BCR	biochemical recurrence
BCRFS	biochemical recurrence-free survival
CI	confidence interval
CR	cribriform
DRE	digital rectal examination
DSD	disease-specific death
GG	Grade Group
GS	Gleason score
HR	hazard ratio
IDC(-P)	intraductal carcinoma of the prostate
ISUP	International Society of Urological Pathology
NSM	Negative surgical margin
OR	odds ratio
PI-RADS	Prostate Imaging Reporting and Data System
PLND	post-operative lymph node dissection
PSA	prostate-specific antigen
PSM	positive surgical margin
RP	radical prostatectomy
WHO	World Health Organization

Curriculum vitae



Eva Hollemans was born on the 9th of December 1990 in Dordrecht. She was always interested in nature and technology and loved her side job as assistant nurse in home care. At high school, she wanted to become a pathologist, the perfect amalgamation between healthcare and science. She graduated high school at the Stedelijk Dalton Lyceum, Dordrecht in 2009 and got accepted for medical school at the Erasmus University in Rotterdam. During her studies, she explored matrix metalloproteinases in ovarian cancer with dr. Vos, which sparked her interest for research. She enrolled the dedicated internship at the Erasmus MC Pathology Department in 2015. Her medical degree was received in January, 2016. After a year of residency, she interrupted her training to fully focus on her PhD. In October 2019 she continued her residency, which she expects to finish in December 2022.

Eva lives in Dordrecht. She likes cats, whisky and ballet.

PhD PORTFOLIO

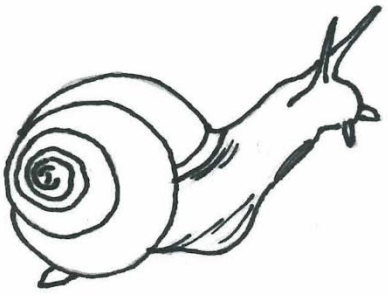
Name PhD student: E. Hollemans	PhD period: 01-04-2017 – 31-10-2020
Erasmus MC Department: Pathology	Promotor(s): Prof. Dr. F. van Kemenade
Research School: Mol Med	Supervisor: Dr. G.J.L.H van Leenders

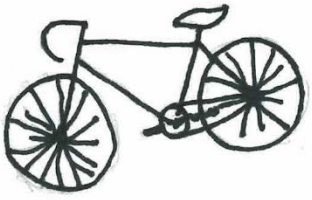
1. PhD training	**Year**	**ECTS**
General courses		
- Statistics: Basic course on 'R'	2017	1.8
- Basic and Translational Oncology	2017	1.8
- Research Integrity	2017	0.3
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2018	1.5
Pathology courses		
- LPAV Cursus (Utrecht, Dermatopathologie)	2018	0.5
- LPAV Cursus (Utrecht, Nefropathologie)	2019	0.5
- LPAV Cursus (Utrecht, Schildklierpathologie)	2019	0.5
- LPAV Cursus (Utrecht, Melanocytaire pathologie)	2019	0.5
- Utrechtse Mamma Cursus	2019	0.3
Seminars and workshops		
- Annual PhD Day	2017-2019	1.2
Oral presentations		
- JNI Labmeetings / Presentations	2017-2019	1
- Urology Labmeetings	2017-2019	5
- Urology Journal Clubs	2017-2019	2
- Molecular Meetings	2017-2019	1
- 25th Meeting EAU Section Urology Research (Athens)	2018	1
Poster presentations		
- European Congress of Pathology (Bilbao)	2018	1
- European Congress of Pathology (Glasgow)	2020	0,5
- 10th European Congress on Urol. Cancers (Amsterdam)	2018	1
(Inter)national conferences		
- European Congress of Pathology (Amsterdam)	2017	1.0
- European Congress of Pathology (Bilbao)	2018	1.5
- 25th Meeting EAU Section Urology Research (Athens)	2018	2.0
- 10th European Multidisciplinary Congress on Urological Cancers (Amsterdam)	2018	1.0
Other		
Rosai session: prostate	2017	0.2

2. Teaching	**Year**	**ECTS**
Lectures and tutoring		
- VO Microscopie van nieren en urinewegen	2017-2020	5
- VO Microscopie van endocriene organen		
- VO Diagnose en stadiumbepaling van kanker		
- VO Microscopie van mnl genitaliën en spermatogenese		
- VO Histopathologie van epitheliale tumoren		
- VO Microscopie van het maagdarmstelsel		
- Clinical Trial Center: Inleiding in de Pathologie	2019	0.5
Other		
- Supervising pathology residents in research	2020	1

Total: 33.6 ECTS

Dankwoord



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Ceterum censeo Carthaginem esse delendam.