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Aims: Gleason pattern 4 (GP4) percentage, invasive cribriform and/or intraductal carcinoma (IC/IDC) and the presence of tertiary Gleason pattern 5 (TP5) in radical prostatectomy (RP) specimens all aid in the risk stratification of Grade Group (GG) 2 prostate cancer patients. However, it is unclear to what extent these pathological features are mutually related and what are their individual values if they are investigated simultaneously. The aims of this study were: (i) to determine the mutual relationships of the GP4 percentage, IC/IDC and TP5 in GG2 RP specimens; and (ii) to assess their prognostic value for biochemical recurrence-free survival (BCRFS).

Methods and results: Of 1064 RP specimens, 472 (44.4%) showed GG2 prostate cancer. Patients with ≥25% GP4 more frequently had IC/IDC (67.0% versus 43.9%; $P < 0.001$) and TP5 (20.6% versus 5.8%; $P < 0.001$) than those with <25% GP4. In unadjusted analysis, an increased GP4 percentage [hazard ratio (HR) 1.3; 95% confidence interval (CI) 1.0–1.6; $P = 0.04$] and IC/IDC (log rank $P < 0.001$) were associated with shorter BCRFS, whereas TP5 ($P = 0.12$) and a dichotomised (<25%, ≥25%) GP4 percentage ($P = 0.10$) were not. In multivariable analysis, IC/IDC was an independent prognostic factor (HR 1.9; 95% CI 1.2–2.9; $P = 0.005$) for BCRFS, whereas a continuous or dichotomised GP4 percentage and TP5 were not independent prognostic factors.

Conclusion: In conclusion, a higher GP4 percentage in RP specimens was associated with more frequent IC/IDC and TP5. IC/IDC was an independent predictor for BCRFS, whereas the GP4 percentage and TP5 were not. These findings underscore the importance of routinely including the presence of IC/IDC in RP pathology reports.

Keywords: cribriform, Gleason score, prostate cancer, quantity, radical prostatectomy, tertiary pattern

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Introduction

The Gleason grading system is one of the cornerstones of prostate cancer (PCa) risk stratification and clinical decision-making. In radical prostatectomy (RP) specimens, the Gleason score/Grade Group (GG) is determined by adding the most common Gleason patterns. Patients with a biopsy Gleason score of 6 (GG1) are often eligible for active surveillance, and those with GG1 disease in RP specimens have a very low, if any, risk of developing metastasis.[1,2] In contrast, patients with biopsy GG3–5 PCa are considered to have aggressive disease and will generally be treated with RP, radiation therapy, and/or hormonal therapy. The clinical outcome of patients with biopsy Gleason score 3 + 4 = 7 (GG2) PCa is highly variable, with an increasing number of patients being considered to be eligible for active surveillance.[3,4]

GG2 PCa is pathologically a heterogeneous disease, with variable Gleason pattern 4 (GP4) percentages and different growth patterns. The risk of biochemical recurrence increases with an increasing GP4 percentage in RP specimens.[5] GP4 includes poorly formed, fused, cribriform and glomeruloid glandular structures. In recent years, cribriform architecture has been associated with biochemical recurrence, metastatic disease, and disease-specific mortality.[1,5–9] Cribriform architecture can occur as an invasive GP4 structure or as intraductal carcinoma of the prostate, in which the malignant proliferation is present within distended pre-existing glands with a basal cell layer. Because invasive cribriform and/or intraductal carcinoma (IC/IDC) is an independent predictor for an adverse clinical outcome, the International Society of Urological Pathology (ISUP) and Genitourinary Pathology Society recommend reporting its presence in biopsy and RP specimens.[10,11] Finally, the presence of minor/tertiary components of Gleason pattern 5 (TP5) constituting <5% of the tumour volume in GG2 RP specimens has been associated with shorter biochemical recurrence-free survival (BCRFS).[12,13]

The GP4 percentage, IC/IDC and TP5 aid in the risk stratification of GG2 PCa patients. Most studies, however, have analysed only one of these pathological features without adjusting for the other prognostic factors. Therefore, at present, it is not clear to what extent these pathological features are mutually related and which of them have independent prognostic value if investigated simultaneously.[10] The aims of our study were: (i) to determine the mutual relationships of the GP4 percentage, IC/IDC and TP5 in GG2 RP specimens; and (ii) to assess their prognostic value for BCRFS.

Materials and methods

PATIENT SELECTION

Patients who had undergone RP for prostatic adenocarcinoma at three medical centres in The Netherlands between 2000 and 2017 were included in this study; 854 patients were operated on at Erasmus MC Cancer Institute, University Medical Centre, Rotterdam, 96 at Leiden University Medical Centre (LUMC), Leiden, and 137 at Antoni van Leeuwenhoek Hospital, the Netherlands Cancer Institute (NKI), Amsterdam. Whereas the operation specimens of Erasmus MC were unselected consecutive samples, those from LUMC and the NKI were selected for the presence of GG3–5 disease in the original pathology report in order to increase the number of high-grade tumours. We excluded patients who had undergone hormonal, radiation and/or viral therapy (n = 23) before operation. RP specimens were fixed in neutral-buffered formalin, after which they were sectioned transversely and embedded in their entirety for diagnostic purposes. All slides were available for pathology review. The institutional Medical Research Ethics Committee approved this study (MEC-2018-1614).

PATHOLOGICAL EVALUATION

All 1064 RP specimens were reviewed in joint sessions by two investigators (E.H. and G.v.L.) blinded to clinical outcome. For each specimen, the following features were recorded: Gleason score and GG according to the 2014 ISUP/2016 World Health Organisation guidelines, pT stage according to the American Joint Committee on Cancer TNM 8th edition, surgical margin status, the presence of IC/IDC, and the percentages of GP4 and TP5. Invasive cribriform and intraductal carcinoma were not distinguished and were grouped for all analyses. We defined the cribriform pattern as a contiguous epithelial proliferation: (i) in which the majority of tumour cells do not contact the surrounding stroma; (ii) with a gland-like space surrounding less than half of the sheet circumference; and (iii) with regular intercellular lumens clearly visible on haematoxylin and eosin-stained sections.[14–17] Specifically regarding point (ii) above, glomeruloid structures and roman bridging were not considered to indicate a cribriform pattern. In cases of multifocality, we only monitored the characteristics of the index tumour defined as the tumour with the highest grade, stage, or volume. In none of the cases was IC/IDC or TP5 absent in the index tumour but present in a non-index lesion. Tertiary patterns

occupying <5% of the tumour volume and IDC were not included in the Gleason score. The GG concordance rates at revision were 88/135 (65.2%) for RP specimens from the NKI and 39/94 (41.5%) for RP specimens from LUMC.

CLINICAL FOLLOW-UP

Clinical follow-up after RP consisted of 6-monthly, and later annual, monitoring of serum prostate-specific antigen (PSA) levels. Biochemical recurrence was defined as PSA levels of ≥0.2 ng/ml measured at two consecutive points in time, at least 3 months apart, with undetectable PSA levels after the operation. Postoperative lymph node and distant metastases were confirmed by biopsy, imaging, and/or multidisciplinary consensus.

STATISTICAL ANALYSIS

Continuous variables were analysed with the Mann–Whitney *U*-test. For comparison of categorical parameters, Pearson's chi-squared (χ^2) test was used, and Fisher's exact test was used in the case of small numbers ($n \leq 20$). Missing PSA values ($n = 27$) were imputed by use of the median PSA value. BCRFS and metastasis-free survival were analysed with the Cox proportional hazards model and visualised by the use of Kaplan–Meier curves. Statistical analyses were performed with SPSS version 25 (IBM, Chicago, IL, USA). Results were considered significant when the two-sided *P*-value was <0.05.

Results

PATIENT CHARACTERISTICS

Of 1064 RP specimens, 472 (44.4%) showed GG2 PCa. The median age at time of operation was 63.8 years [interquartile range (IQR) 60.0–68.0 years], and the median serum PSA level was 11.0 ng/ml (IQR 6.0–12.9 ng/ml). The pathological tumour stages were T2 in 268 (56.8%) patients, T3a in 169 (35.8%) patients, and T3b in 35 (7.4%) patients. Positive surgical margins were present in 156 (33.1%) cases. Of 249 (52.8%) patients who had undergone pelvic lymph node dissection, 13 (5.2%) had lymph node metastasis. The median GP4 percentage was 20.0% (IQR 10.0–30.0%); 278 (58.9%) patients had GP4 pecentages in the range 5–24%, and 194 (41.1%) had GP4 percentages in the range 25–49%. IC/IDC was observed in 252 (53.4%) patients, and TP5 in 56 (11.9%) patients. The median clinical follow-up was 38 months (IQR 10.4–77.5 months). In total 107 (22.7%) GG2 patients developed postoperative biochemical recurrence after a median of 24 months, and 18 (3.8%) developed postoperative metastasis.

GP4 PERCENTAGES

The clinicopathological features of GG2 patients stratified for 5–4% (278/472; 58.9%) and 25–49% (194/472; 41.1%) are shown in Table 1. TP5 was observed significantly ($P < 0.001$) more often in patients with ≥25% GP4 (40/194; 20.6%) than in those with <25% GP4 (16/278; 5.8%). Also, IC/IDC was observed significantly more often in patients with ≥25% GP4 (130/194; 67.0%) than in those with <25% GP4 (122/278; 43.9%; $P < 0.001$). Patients with ≥25% GP4 more frequently had extraprostatic extension (pT3; 95/194; 49.0%) and were older than those with <25% GP4 (109/278; 39.2%; $P = 0.01$). Serum PSA levels, pelvic lymph node metastasis and

Table 1. Clinicopathological characteristics of Grade Group 2 patients ($n = 472$) stratified for Gleason pattern 4 (GP4) percentages of 5–24% and 25–49%

	<25% GP4 *N* = 278	≥25% GP4 *N* = 194	*P*-value
Age (years), median (IQR)	64.0 (59.1–67.8)	64.8 (61.5–68.7)	0.03
PSA (ng/ml), median (IQR)	10.9 (6.0–13.0)	8.3 (6.0–12.4)	0.72
pT stage, *n* (%)			0.01
pT2	169 (60.8)	99 (51.0)	
pT3a	96 (34.5)	73 (37.6)	
pT3b	13 (4.7)	22 (11.4)	
Positive surgical margin, *n* (%)	93 (33.5)	63 (32.5)	0.82
pN stage *n* (%)			0.29
pN0	141 (50.7)	108 (55.7)	
pN1	6 (2.2)	7 (3.6)	
pNx	131 (47.1)	79 (30.7)	
IC/IDC	122 (43.9)	130 (67.0)	<0.001
TP5	16 (5.8)	40 (20.6)	<0.001

IC/IDC, invasive cribriform and/or intraductal carcinoma; IQR, interquartile range; PSA, prostate-specific antigen; TP5, tertiary Gleason pattern 5.

surgical margin positivity were not significantly different between the groups. Postoperative biochemical recurrence occurred in 56 of 278 (20.1%) men with <25% GP4 and in 51 of 194 (26.3%) patients with ≥25% GP4. Univariate Cox regression analysis revealed that the GP4 percentage GP4 as a continuous variable had prognostic value for BCRFS [hazard ratio (HR) 1.3; 95% confidence interval (CI) 1.0–1.6; $P = 0.04$]. BCRFS was not statistically different when the GP4 percentage was dichotomised as <25% and ≥25% (log-rank $P = 0.10$; Figure 1A). Thirteen (6.7%) patients with ≥25% GP4 developed postoperative metastasis, as compared with five (1.8%) of those with <25% GP4 (log rank; $P = 0.003$).

CRIBRIFORM ARCHITECTURE

The number of GG2 patients with IC/IDC was 252 (53.4%) (Table 2). The GP4 percentages were 25.0% (IQR 20.0–33.7%) in patients with IC/IDC and 15.0% (IQR 10.0–30.0%) in those without ($P < 0.001$). TP5 was observed in 34 (13.5%) IC/IDC-positive and 22 (10.0%) IC/IDC-negative samples ($P = 0.24$). The median PSA level of the patients with IC/IDC was 12.1 ng/ml (IQR 6.3–13.5 ng/ml), which was significantly higher than that of those without IC/IDC (8.0 ng/ml, IQR 5.6–11.1 ng/ml; $P = 0.02$). Patients with IC/IDC more frequently had extraprostatic extension (126/252; 50.0% versus 78/220; 35.4%; $P < 0.001$), and positive surgical margins (97/252; 38.5% versus 59/220; 26.8%; $P = 0.007$) than those without. All 13 patients with pelvic lymph node metastasis had IC/IDC within their RP specimen; no metastases occurred in patients without IC/IDC ($P < 0.001$). BCRFS in patients with IC/IDC (73/252; 29.0%) was shorter (log rank $P < 0.001$) than in those without (34/220; 15.5%) (Figure 1B). Eighteen (7.1%) patients with IC/IDC developed postoperative metastasis, whereas none of 220 patients without IC/IDC did so (log rank $P < 0.001$).

TP5

The median GP4 percentage of patients with TP5 (30.0%, IQR 20.0–40.0%) was significantly ($P < 0.001$) higher than that of those without TP5 (20.0%, IQR 10.0–30.0%). PSA levels (9.2 ng/ml, IQR 7.3–16.4 ng/ml) were also higher ($P = 0.03$) when TP5 was present than when it was not (8.3 ng/ml, IQR 5.9–12.1 ng/ml). IC/IDC was observed in 34 of 56 (60.7%) patients with TP5 and in 218 of 416 (52.4%) of those without ($P = 0.24$). Age, pT stage, surgical margin status and pelvic lymph node metastasis were not significantly different between the two groups (Table 3). Sixteen of 56 (28.6%) patients with TP5 developed biochemical recurrence as compared with 91 of 416 (21.9%) of those without; BCRFS was not statistically significant in this cohort (Figure 1C; log rank $P = 0.12$). Four of 56 (7.1%) patients with TP5 developed postoperative metastasis as compared with 14 of 416 (3.4%) without (log rank $P = 0.04$).

MULTIVARIABLE ANALYSIS

In Cox regression analysis adjusted for routine clinicopathological parameters, i.e. the GP4 percentage, IC/IDC, and TP5, the presence of IC/IDC was an independent prognostic factor (HR 1.7; 95% CI 1.1–2.6; $P = 0.02$) for BCRFS (Table 4). The GP4 percentage, included either as a continuous parameter (HR 1.0; 95% CI 0.8–1.3; $P = 0.64$) or a categorical parameter (5–24%; 25–49%) (HR 1.1; 95% CI 0.7–1.6; $P = 0.71$), and TP5 (HR 0.7; 95% CI 0.4–1.3;

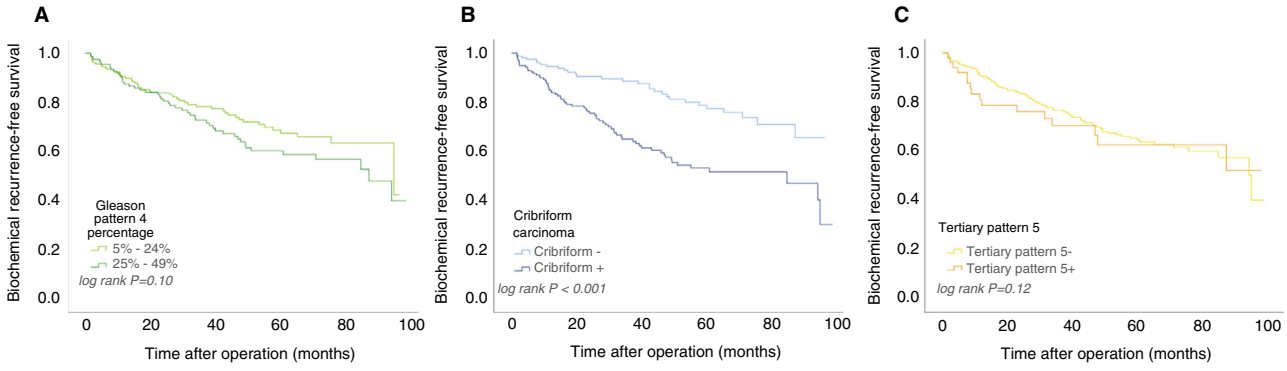

Figure 1. Biochemical recurrence-free survival in patients with Grade Group 2 prostate cancer, stratified for: (**A**) Gleason pattern 4 (log-rank $P = 0.10$); (**B**) invasive cribriform and/or intraductal carcinoma (log-rank $P < 0.001$); and (**C**) tertiary Gleason pattern 5 (log-rank $P = 0.12$).

Table 2. Clinicopathological characteristics of Grade Group 2 patients ($N = 472$) stratified for the presence of invasive cribriform and/or intraductal carcinoma (IC/IDC)

	IC/IDC-negative $N = 220$	IC/IDC-positive $N = 252$	*P*-value
Age (years), median (IQR)	63.1 (59.4–68.1)	64.7 (60.3–67.9)	0.22
PSA (ng/ml), median (IQR)	8.0 (5.6–11.1)	12.1 (6.3–13.5)	0.02
pT stage, *n* (%)			<0.001
pT2	142 (64.5)	126 (50.0)	
pT3a	72 (32.7)	97 (38.5)	
pT3b	6 (2.7)	29 (11.5)	
Positive surgical margin, *n* (%)	59 (26.8)	97 (38.5)	0.007
pN stage, *n* (%)			<0.001
pN0	102 (46.4)	147 (58.3)	
pN1	0 (0.0)	13 (100.0)	
pNx	118 (53.6)	92 (36.5)	
% GP4 (continuous), median (IQR)	15.0 (10.0–30.0)	25.0 (20.0–33.7)	<0.001
% GP4 (categorical), *n* (%)			<0.001
5–24	156 (70.9)	122 (48.4)	
25–49	64 (29.1)	130 (51.6)	
TP5, *n* (%)	22 (10.0)	34 (13.5)	0.24

GP4, Gleason pattern 4; IQR, interquartile range; PSA, prostate-specific antigen; TP5, tertiary Gleason pattern 5.

Table 3. Clinicopathological characteristics of Grade Group 2 patients ($N = 472$) stratified for tertiary Gleason pattern 5 (TP5)

	TP5 absent $N = 416$	TP5 present $N = 56$	*P*-value
Age (years), median (IQR)	64.6 (56.7–68.1)	64.4 (60.6–67.9)	0.63
PSA (ng/ml), median (IQR)	8.3 (5.9–12.1)	9.2 (7.3–16.4)	0.03
pT stage, *n* (%)			0.06
pT2	243 (58.4)	25 (44.6)	
pT3a	145 (34.9)	24 (42.9)	
pT3b	28 (6.7)	7 (12.5)	
Positive surgical margin, *n* (%)	139 (33.2)	17 (30.4)	0.76
pN, *n* (%)			0.10
pN0	220 (52.9)	29 (51.8)	
pN1	9 (2.2)	4 (7.1)	
pNx	187 (45.0)	23 (41.1)	
% GP4 (continuous), median (IQR)	20.0 (10.0–30.0)	30.0 (20.0–40.0)	<0.001
% GP4 (categorical), median (IQR)			<0.001
5–24	262 (63.0)	16 (28.6)	
25–49	154 (37.0)	40 (71.4)	
IC/IDC	218 (52.4)	34 (60.7)	0.24

GP4, Gleason pattern 4; IC/IDC, invasive cribriform and/or intraductal carcinoma; IQR, interquartile range; PSA, prostate-specific antigen.

$P = 0.33$) did not have prognostic value in multivariable analysis (Table 4). This outcome was unchanged when PSA was excluded from multivariable analysis. In total, 18 of 472 (3.8%) patients developed postoperative metastasis after a median of 58 months. All of these 18 patients had IC/IDC in RP specimens, 13 of 18 (72.2%) had ≥25% GP4, and four of 18 (22.2%) had TP5. The number of metastatic events was too small for further analysis.

Discussion

The GP4 percentage, IC/IDC and the presence of TP5 have added value in the risk stratification of GG2 PCa patients. As these pathological features have mostly been assessed individually, it is not yet clear to what extent they are mutually related and what their impact is if the other features are taken into account. In this study, we demonstrate that the GP4 percentage, the presence of IC/IDC and TP5 are mutually related in GG2 RP specimens. In multivariable analysis, IC/IDC was an independent prognostic parameter for BCRFS, whereas the GP4 percentage and TP5 were not. These findings suggest that the clinical value of the GP4 percentage and TP5 can, at least in part, be attributed to their association with IC/IDC, and underscore the importance of routinely reporting the presence of IC/IDC.

Table 4. Cox regression analysis of biochemical recurrence-free survival (BCRFS) in Grade Group 2 prostate cancer patients

	Univariate analysis			Multivariable analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
PSA*	1.6	1.3–1.9	<0.001	1.2	1.0–1.5	0.03
pT stage						
pT2	Ref.			Ref.		
pT3a	1.7	1.1–2.7	0.008	1.4	0.9–2.1	0.17
pT3b	4.9	2.8–8.5	<0.001	2.0	1.0–3.8	0.04
Positive surgical margin	2.5	1.7–3.7	<0.001	2.0	1.3–3.0	0.001
pN stage						
pN0	Ref.			Ref.		
pN1	12.0	5.9–24.2	<0.001	6.7	2.9–15.1	<0.001
pNx	0.5	0.3–0.8	0.004	0.7	0.4–1.1	0.09
% GP4 (continuous)*	1.3	1.0–1.6	0.04	1.0	0.8–1.3	0.64
IC/IDC	2.4	1.6–3.6	<0.001	1.7	1.1–2.6	0.02
TP5	1.5	0.9–2.6	0.12	0.7	0.4–1.3	0.33

CI, confidence interval; GP4, Gleason pattern 4; HR, hazard ratio; PSA, prostate-specific antigen; TP5, tertiary Gleason pattern 5.
*2 log.

Although several studies have shown prognostic value for the GP4 percentage, IC/IDC, and TP5, only a few have analysed these parameters simultaneously. For instance, Choy *et al.* showed that both cribriform architecture and a higher GP4 percentage in RP specimens were independently associated with lower 5-year BCRFS in 350 combined GG2 and GG3 patients.[5] Although their finding regarding cribriform carcinoma is in line with our study, the independent value of the GP4 percentage is not. A possible explanation is that we included the GP4 percentage as a continuous variable, whereas Choy *et al.* categorised it as 1–20%, 21–50%, 51–70%, and >70%. Dichotomisation of continuous variables may, however, lead to bias. Furthermore, Choy *et al.* included patients with 1–5% GP4, whereas we excluded those as being GG1 with tertiary GP4. As patients with 1–5% GP4 had the longest BCRFS in their study, the inclusion of this subgroup might have affected the outcome of the multivariable analysis. Kweldam *et al.* found that IC/IDC was more common in GG2 biopsies with a higher GP4 percentage.[18] In multivariable analysis of postoperative BCRFS, IC/IDC was an independent factor, whereas GP4 percentage was not, which is in line with the current RP study. Finally, in an RP cohort of 8057 GG2 patients, Sauter *et al.* showed that both GP4 percentage and the presence of TP5 had additional value in predicting BCRFS.[12] The frequency of TP5 in the study of Sauter *et al.* (742/8057; 9.2%) was comparable to ours (56/472; 11.9%). In our study, patients with TP5 had lower BCRFS rates than those without, but this difference was not statistically significant. A possible explanation might be our relatively small study population, which limited powerful statistical analysis of subtle differences. As Sauter *et al.* did not include IC/IDC, the impact of this parameter in their large cohort is unknown.

Our findings corroborate the adverse impact of the presence of IC/IDC in PCa patients. An increased GP4 percentage and the presence of TP5 have also been identified as pathological parameters associated with an adverse outcome. In our study, we showed that these three parameters are related. Among GG2 patients with ≥25% GP4, 67% had IC/IDC and 21% had TP5, whereas these figures were 44% and 6%, respectively, in patients with <25% GP4. Although TP5 was observed slightly more often in patients with IC/IDC than in those without, this difference was not statistically significant, which may due to the relatively small sample size. Validation of these mutual relationships and adjusted analysis in future studies will be important to confirm the current data, and to

obtain comprehensive insights into the independent clinical impacts of these pathological features.

Interobserver variability is one of the most important caveats regarding PCa grading. Two studies showed that reproducibility of cribriform architecture is substantially better than that of other growth patterns.[19,20] In an interobserver study of 80 RP slides, the reproducibility of assigning TP5 was lower than those of IC/IDC and the GP4 percentage.[21] As far as we know, no specific interobserver studies have yet been performed on tertiary patterns. We hypothesise that the reproducibility of assigning TP5 is low because it can be overlooked or interpreted as poorly formed GP4. Also, estimation of minor pattern volumes might be troublesome. In a Gleason score 3 + 4 = 7 tumour, estimation of a minor TP5 component as 6% would imply upgrading to a Gleason score of 3 + 5 = 8, whereas it would be regarded as a Gleason score 3 + 4 = 7 tumour with TP5 if the minor component was assessed as 4%.[10,22] Also, any IC/IDC presence, even if its volume is limited, is associated with an adverse outcome, facilitating its use in clinical practice.[23,24]

The presence of a cribriform pattern is most relevant in patients with intermediate-risk PCa on biopsy, in whom it can directly affect eligibility for active surveillance. Although a cribriform pattern does not generally have an influence on postoperative therapy, the very low risk of metastasis in GG2 patients without a cribriform pattern might facilitate the communication of RP outcome and reduce the intensity of long-term follow-up.

To our knowledge, this is the first study addressing the mutual relationships of the GP4 percentage, IC/IDC and TP5 in GG2 RP specimens. Strengths of this study are the inclusion of a relatively large RP cohort and detailed monitoring of pathological parameters. The retrospective design and relatively short follow-up period are general weaknesses of our study. Although all parameters were individually associated with metastasis-free survival, multivariable analysis was not possible, owing to the low number of events. In that sense, it is of interest to note that GG2 patients without IC/IDC in RP specimens had comparable excellent metastasis-free survival to that of GG1 patients in the same cohort.[25]

In conclusion, the GP4 percentage, the presence of IC/IDC and TP5 are mutually related in GG2 RP specimens. Although both the GP4 percentage and IC/IDC were prognostic for BCRFS in univariate analysis, IC/IDC was the only independent factor that was prognostic for BCRFS in adjusted analysis; the GP4 percentage and TP5 were not. This outcome underscores the importance of explicitly including the presence or absence of a cribriform pattern in RP pathology reports.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

N. Seyrek and E. Hollemans performed the research. N. Seyrek analysed the data and wrote the manuscript. S. Osanto, R. C. M. Pelger, H. G. van der Poel, E. Bekers, C. H. Bangma, J. Rietbergen and M. J. Roobol included patients with clinical follow-up. I. G. Schoots and G. J. L. H. van Leenders supervised the analysis and manuscript drafting. G. J. L. H. van Leenders designed the study.

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